MP Diagnostics HTLV Blot 2.4

BLA 125475

Briefing Document for the Blood Products Advisory Committee

Meeting Date: November 1, 2013

THE CONTENTS OF THIS BRIEFING PACKAGE ARE FULLY RELEASABLE

TABLE OF CONTENTS

2. LIST OF TABLES

3. LIST OF FIGURES

4. ABBREVIATIONS AND DEFINITIONS OF TERMS

ASTPHLD	Association of State and Territorial Public Health Directors
ATL	Adult T-cell Leukemia/Lymphoma
BLA	Biologics License Application
bp	Base Pairs
CDC	Center for Disease Control and Prevention
CDPHL	California Department of Public Health Laboratory
ChLIA	Chemiluminescent Immunoassay
CI	Confidence Interval
COV	Cut-off Value
DB	Diagnostics Biotechnology Pte Ltd
DNA	Deoxyribonucleic Acid
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
Env	A viral protein that serves to form the viral envelope; resides in lipid layer; determines viral tropism. The gene is denoted by *env.*
FDA	Food and Drug Administration
Gag	A polyprotein (group antigens); processed to matrix and other core proteins that determine retroviral core. The gene is denoted by *gag.*
GD21	HTLV immunodominant recombinant *env* epitope derived from a truncated portion of the HTLV-I p21e gene product with demonstrated immunoreactivity to HTLV type 1 and type 2
GLD	Genelabs Diagnostics Pte Ltd
GLT	Genelabs Technologies, Inc
GST	Glutathione S-transferase
HAM/TSP	HTLV-I Associated Myelopathy/Tropical Spastic Paraparesis
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus

HGIP	HTLV Gag Indeterminate Profile
HIV	Human Immunodeficiency Virus
HTLV	Human T-cell Lymphotropic Virus
HTLV-I	Human T-cell Lymphotropic Virus, type 1
HTLV-II	Human T-cell Lymphotropic Virus, type 2
IFA	Immunofluorescence Antibody Assay
IFU	Instructions for Use
IND	Investigational New Drug/Investigational New Device
IUO	Investigational Use Only
IVD	In vitro diagnostics
LIA	Line Immunoassay
LDT	Laboratory Developed Tests
MDUFA	Medical Device User Fee Act
NIH	National Institutes of Health
p21e	HTLV immunodominant recombinant *env* epitope derived from the HTLV-I gp21 gene product
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PI	Package Insert
RIPA	Radioimmunoprecipitation Assay
RNA	Ribonucleic Acid
RR	Repeat Reactive
SDS-PAGE	Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis
TOR	Test of Record
U.S.	United States
USPHS	United States Public Health Services

5. EXECUTIVE SUMMARY

a) Need for HTLV Confirmatory Testing

Human T-cell Lymphotropic Viruses (HTLVs) are pathogenic retroviruses that may cause severe hematological and neurological diseases in infected individuals. The HTLV family comprises two dominant viral types, HTLV type 1 and type 2, which are type C human oncoviruses with single stranded ribonucleic acid (RNA) genomes approximately 9800 base pairs (bp) in length. HTLVs include *gag* and *env* genes; *gag* genes encode structural core proteins p19 and p24, while *env* genes encode envelope protein gp61/68, a precursor to *env* proteins gp46 and gp21. Infection with the HTLV type 1 virus (HTLV-I) is associated with Adult T-cell Leukemia/Lymphoma (ATL), HTLV-I Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) and HTLV-associated uveitis. Infection with the HTLV type 2 virus (HTLV-II) is associated with benign lymphocytosis and chronic neurodegenerative disease. Epidemiological studies of HTLV indicate a mixed prevalence of HTLV-I and HTLV-II in high-risk populations in the United States (U.S.), with global prevalence varying by population. HTLV is a blood borne virus, and as such, transmission occurs from mother to child, by sexual contact, by sharing contaminated needles, and through blood transfusion.

Screening of the blood donor population for HTLV has been mandatory in the U.S. since 1988. Currently, there are two licensed screening assays for HTLV, the Abbott PRISM HTLV-I/HTLV-II and the AVIOQ HTLV-I/II Microelisa System. As screening assays, these products identify donor specimens that are presumptive seropositive but do not confirm or differentiate. A more specific supplemental assay is required for confirmation of HTLV viral infection as well as discrimination of HTLV viral type, needed for donor counseling and notification. Currently, there are no licensed supplemental assays available in the U.S. to meet the need for HTLV confirmation and viral type differentiation. The MP Diagnostics HTLV Blot 2.4 is a qualitative enzyme immunoassay (EIA), intended as an HTLV supplemental assay to confirm and differentiate antibodies to HTLV-I/II, and designed to meet the needs of the blood bank industry for supplemental testing.

b) Overview of the MP Diagnostics HTLV Blot 2.4 Product Development

The MP Diagnostics HTLV Blot 2.4 has been available on the market in various iterations since 1985. The original product, the HTLV-I Blot 1.2, was developed under Diagnostic Biotechnology Pte Ltd (DB) in 1985, and contained native proteins derived from an HTLV-I viral lysate only. The HTLV-I Blot 2.0, developed in 1987, added recombinant antigen p21e, an early recombinant derived from the gp21 envelope protein. The development of the HTLV Blot 2.2, with the addition of HTLV-I type specific recombinant protein MTA-4, soon followed in 1988. The next significant development occurred in 1991 with the release of the HTLV Blot 2.3, which included the HTLV-II type specific recombinant antigen K-

55, replaced MTA-4 with MTA-1, and included an anti-human IgG band for assay validity and sample addition verification. The HTLV Blot 2.4 quickly followed in 1993, released under the company name of Genelabs Diagnostics Pte Ltd (GLD); in the HTLV Blot 2.4, the early recombinant p21e was replaced with the more specific GD21 protein. In 2004, the interpretationcriteria for the HTLV Blot 2.4 were revised to follow to the guidelines still used with this product today, and the product CE marked. The MP Diagnostics HTLV Blot 2.4 has been available since 1993, and has a history of longevity and quality.

c) HTLV Blot 2.4 Product Information

The MP Diagnostics HTLV Blot 2.4 is a qualitative EIA intended for confirming and differentiating the presence of antibodies to HTLV-I and HTLV-II in human serum or plasma. It is intended for use as a more specific supplemental test for human serum or plasma samples with repeatedly reactive results by an FDA-approved HTLV-I/II screening test.

The MP Diagnostics HTLV Blot 2.4 is intended as a supplemental test to confirm the presence of anti-HTLV-I/II antibodies and differentiate between HTLV type-I, HTLV type-II and HTLV-I/II dual infections for donor notification and counseling. The possible serological profiles defined by the HTLV Blot 2.4 include the following: HTLV-I Seropositive, HTLV-II Seropositive, HTLV-I/II Seropositive, Seronegative, and Indeterminate.

The MP Diagnostics HTLV Blot 2.4 uses a combination of HTLV-I/II genetically engineered proteins (recombinant proteins) and HTLV-I viral proteins derived from native, inactivated viral particles (viral lysate). The differentiation between HTLV-I and HTLV-II is accomplished with rgp46-I (MTA-1), an unique HTLV-I envelope recombinant protein, and rgp46-II (K-55), a unique HTLV-II envelope recombinant protein. Both proteins are derived from the central region of the external glycoprotein, gp46, of the respective HTLV-I and HTLV-II viral types. GD21, a common yet specific HTLV-I and HTLV-II epitope envelope recombinant protein (rgp21), is also used to enhance the sensitivity of envelope antibody detection. The antigenicity exhibited by these proteins is either common to HTLV-I and HTLV-II antibodies, or type specific to one of the two viruses to allow confirmation and discrimination in a single assay.

d) HTLV Blot 2.4 Interpretation Criteria

The HTLV Blot 2.4 interpretation criteria were revised in 2004 during the CE mark phase, and have remained consistent. Revisions in 2004 to the interpretative criteria included HTLV *Gag* Indeterminate Profiles (HGIP), combinations of *gag* proteins without p24, and single *gag* proteins including p24 are seronegative. Additionally, discrimination of HTLV viral type using the relative intensities of p19 and

p24 in the absence of immunoreactivity to a type specific recombinant was introduced. The HTLV Blot 2.4 Interpretation Criteria have been established as follows:

HTLV Blot 2.4 Interpretation Criteria

Seronegative	• No reactivity to HTLV specific proteins; or • Any combination of *gag* proteins excluding p24 (i.e. p19, p26, p28, p32, p36, p53); or • Any single *gag* protein, inclusive of p24
HTLV-I Seropositive	• Reactivity to p19, GD21 ***and*** rgp46-I: or • Reactivity to p19, p24 ***and*** GD21, with reactivity to p19 greater than or equal to p24
HTLV-II Seropositive	• Reactivity to p24, GD21 ***and*** rgp46-II: or • Reactivity to p19, p24 ***and*** GD21, with reactivity to p24 greater than p19
HTLV-I/II Seropositive	• Reactivity to GD21, p19, p24, rgp46-II ***and*** rgp46-I
Indeterminate	• Reactivity to HTLV specific bands that do not meet the criteria for HTLV-I seropositive, HTLV-II seropositive, HTLV-I/II seropositive or seronegative

e) U.S. Clinical Trial Data

1) Sensitivity in a Known Positive Population

The performance of the HTLV Blot 2.4, including that of the Interpretation Criteria, was evaluated in clinical studies on blood donor populations by comparison of HTLV Blot 2.4 results with those obtained from matched plasma specimens using the California Department of Public Health Laboratories (CDPHL) HTLV Supplemental Algorithm. The validity of the HTLV Blot 2.4 assay was evaluated using two population types: 1) specimens that had screened HTLV non-reactive using a licensed screening assay; and 2) specimens that screened repeatedly reactive (RR) on two screening assays but were unconfirmed positives. A sensitivity estimation study was planned that would assess the performance of the HTLV 2.4 in an HTLV known positive population. Additionally, the reproducibility of the assay was evaluated by testing aliquots of a 3-member HTLV panel over multiple lots, clinical testing sites, operators, and replicates. The results of the reproducibility study demonstrated that the performance of the HTLV Blot 2.4 was reproducible within assay, within site, within lot and within panel member.

The sensitivity of the HTLV Blot 2.4 was evaluated using 200 repository specimens from a well-characterized, known positive population at three geographically distinct clinical testing sites. These specimens were from deferred donors that had previously tested repeatedly reactive using a licensed screening assay in conjunction with research use HTLV supplemental testing, including immunofluorescence assay (IFA), Western blot and radioimmunoprecipation assay (RIPA). The sensitivity results are summarized in **Table 01** below.

Table 01: HTLV Blot 2.4 Sensitivity

MP Diagnostics HTLV Blot 2.4	CDPHL HTLV Supplemental Algorithm			
	Positive	**Indeterminate**	**Negative**	**Total**
Positive	190	1	4	195 (97.5%)
Indeterminate	3	0	1	4 (2.0%)
Negative	0	0	1[a]	1 (0.5%)
Total	193 (96.5%)	1 (0.5%)	6 (3.0%)	200

[a] One sample was negative by both assays

A greater number of known positive specimens were identified as positive by the MP Diagnostics HTLV Blot 2.4 than by the CDPHL HTLV Algorithm (195 versus 193, respectively). Additionally, the MP Diagnostics HTLV Blot 2.4 identified more samples as reactive (i.e. positive or indeterminate) than the CDPHL HTLV Algorithm (199 versus 194, respectively). Of the four samples identified as negative by the CDPHL HTLV Algorithm, all of these were identified as positive by the MP Diagnostics HTLV Blot 2.4, by the presence of, at minimum, two recombinant envelope proteins and one gag protein. In this study, the calculated sensitivity of the MP Diagnostics HTLV Blot 2.4 was 97.5% (195/200) with a 95% CI of 94.26 - 99.18% and a P-value of 0.48. The indeterminate rate for this study was 2% (4/200).

2) Percent Negative Agreement in an HTLV Screening Non-reactive Population

A total of 200 repository specimens from a normal blood donor population were evaluated at three geographically distinct clinical testing sites. These specimens were from blood donors that had tested HTLV non-reactive using a licensed HTLV screening assay. Results from the HTLV screening non-reactive population are summarized in **Table 02** below.

Table 02: HTLV Blot 2.4 Percent Negative Agreement

MP Diagnostics HTLV Blot 2.4	CDPHL HTLV Supplemental Algorithm			
	Positive	**Indeterminate**	**Negative**	**Total**
Positive	0	0	0	0 (0%)
Indeterminate	0	0	31	31 (15.5%)
Negative	0	0	169	169 (84.5%)
Total	0 (0%)	0 (0%)	200 (100%)	200

The MP Diagnostics HTLV Blot 2.4 did not identify any of the HTLV screening negative specimens as positive. Thirty-one specimens were indeterminate by the MP Diagnostics HTLV Blot 2.4, resulting in an indeterminate rate of 15.5% (31/200). Of these 200 specimens tested by the CDPHL HTLV Algorithm, 15 were repeatedly reactive by ELISA. The majority of these repeatedly reactive samples were resolved at the Western blot stage of the CDPHL HTLV Algorithm, based on non-reactivity from both the IFA and Western blot. Two of these 15 samples, however, showed reactivity with the p21e protein on the Western blot and were subjected to additional testing using RIPA. A non-reactive result on

the RIPA for these two specimens resulted in an overall call of negative by the CDPHL Algorithm. All but one of these 15 specimens was negative by a single MP Diagnostics HTLV Blot 2.4 assay. In this study, the percent negative agreement of the MP Diagnostics HTLV Blot 2.4 is 84.5% with a 95% CI of 78.73 – 89.22%.

3) Percent Positive Agreement in an HTLV Screening Repeatedly Reactive Population

A total of 200 repository specimens from a normal blood donor population were evaluated at three geographically distinct clinical testing sites. These specimens were from blood donors that had tested HTLV repeatedly reactive (RR) using a licensed HTLV screening assay, followed by a second RR result using a unlicensed HTLV screening assay. Results from the HTLV screening RR population are summarized in **Table 03** below.

Table 03: HTLV Blot 2.4 Percent Positive Agreement

MP Diagnostics HTLV Blot 2.4	**CDPHL HTLV Supplemental Algorithm**			
	Positive	**Indeterminate**	**Negative**	**Total**
Positive	0	0	8	8 (4.0%)
Indeterminate	0	0	90	90 (46.0%)
Negative	0	3	99	102 (51.0%)
Total	0 (0%)	3 (1.5%)	197 (98.5%)	200

The HTLV Blot 2.4 identified eight out of the 200 samples as HTLV seropositive. Comparatively, the CDPHL HTLV Supplemental Algorithm did not identify any samples as positive.

f) Risk/Benefit Analysis

The expected benefit from commercialization of a licensed HTLV confirmatory assay is twofold: the correct, reliable interpretation of RR specimens for the purpose of donor counseling and treatment; and unnecessary donor loss (under donor reentry) due to diminished specificity of HTLV screening assays.

The results of the clinical studies strongly suggest that both benefits will be actualized from the licensure of the HTLV Blot 2.4. The HTLV Blot 2.4 did not identify any sample as positive in the HTLV screening non-reactive population, so the assay does not misinterpret a result. Additionally, the sensitivity of the HTLV Blot 2.4 in the known positive population was 97.5%, so the HTLV Blot 2.4 is able to identify seropositive samples. Finally, the HTLV Blot 2.4 was able to identify positive samples within the RR population that were missed by the other assay, thereby demonstrating the sensitivity of the assay.

g) Conclusions

The HTLV Blot 2.4 is a supplemental Western blot assay intended for confirmation and discrimination. The ability of the assay to differentiate between viral type infection is amplified through the use of type specific recombinant antigens and a common, yet specific, recombinant. Additionally, use of viral lysate allows for differentiation of HTLV viral type using p19 and p24 in the absence of a type specific recombinant.

6. HTLV OVERVIEW

a) Disease Summary

Human T-cell Lymphotropic Viruses (HTLVs) are pathogenic retroviruses that may cause severe hematological and neurological diseases in infected individuals. The HTLV family comprises two well-studied members, HTLV-I and HTLV-II, as well as two newly discovered members, HTLV-3 and HTLV-4. HTLV-I is known as the etiological agent of adult T-cell leukemia / lymphoma (ATL), HTLV-associated myelopathy / tropical spastic paraparesis (HAM / TSP), and HTLV-associated uveitis. HTLV-II infection has also been associated with leukemia, neurological and pulmonary diseases, although it is less pathogenic than HTLV-I. Several lines of molecular evidences suggest that HTLV-3 possesses some of the HTLV-I properties, although little is known about the pathogenicity of HTLV-3. Studies of the geographic distribution of HTLV-I infection reveal that the virus is highly prevalent in Japan, Africa, Caribbean Islands, and South America. Recent epidemiological studies in the U.S. and Europe confirm the presence of a mixed prevalence of both HTLV-I and HTLV-II among different high risk populations such as intravenous drug users (IDU). The viruses can be transmitted through sexual contact, through contaminated blood products, and from mother to child via breast-feeding.

HTLV-I and -II are type C human oncoviruses with single stranded RNA genomes that are approximately 8,900 base pairs in length. The HTLV-I and II genomes include gag and env genes that encode structural core proteins p19 and p24, as well as envelope proteins gp46 and p21e[1]. Like other human retroviruses, the HTLV-I and -II pol gene encodes a reverse transcriptase to allow transcription of its RNA genome into a complementary DNA strand, which is then incorporated into the host genome by a pol encoded integrase.

HTLV-I and -II infection is generally diagnosed by antibody tests (ChLIA, Western blot, immunofluorescence (IFA)). Due to the inclusion of cross-reactive antigens, most assays detect both HTLV-I and HTLV-II antibody, although sensitivity for HTLV-II may be lower[2]. Type-specific natural or recombinant envelope peptides in IFA or Western blot format permit the differentiation of HTLV-I from HTLV-II antibodies[3]. The two virus types may also be distinguished by polymerase chain reaction (PCR) or in-situ hybridization directed at specific HTLV-I and -II proviral DNA or RNA sequences[4]. Quantitative PCR studies have also determined that the proviral DNA load in both HTLV-I and -II ranges from approximately 10^{-4} to 10^{-1} per peripheral blood mononuclear cell[5,6].

HTLV-I is endemic at levels up to five percent of the general population in central Africa, several Caribbean basin and South American countries, and in southern Japan[7]. Transmission is from mother to child, predominantly by breast-feeding, by sexual intercourse predominantly in the male-to-female direction, and via parenteral exposure by blood transfusion or needle-sharing. In the United States, blood

donor HTLV-I seroprevalence is about one per ten thousand and risk factors include maternal or sexual links to HTLV-I-endemic areas; whereas HTLV-II seroprevalence is about two per ten thousand and predominant risk factors are injection drug use (IDU) and sexual contact with an IDU[8,9]. De The has estimated that there may be as many as ten to twenty million persons with HTLV-I infection in the world; a more conservative estimate might be between one to five million[10].

HTLV-II is endemic in certain North[11], Central[12] and South[13, 14] American Indian tribes, with some of the highest seroprevalence values (up to fifty percent) documented in tribes with the least contact with contemporary civilization, such as the Brazilian Kayapo. This led to the hypothesis that HTLV-II was already endemic in these tribes before they migrated across the Bering land bridge over ten thousand years ago. A single report of HTLV-II among Mongolians has not been supported by other studies of the same population[15]. However, clusters of HTLV-II infection have been conclusively demonstrated among isolated Pygmy tribes in central Africa[16, 17]. Genetic similarities between Pygmy and Native American HTLV-I isolates have not been explained[18-20].

An early study which differentiated HTLV-I from HTLV-II using a competitive HTLV-I and -II ELISA technique reported a high seroprevalence of HTLV-II and HTLV-I among IDU in the New Jersey area[21]. In New Orleans, approximately twenty-five percent of IDU tested were HTLV-II positive by PCR and another two percent were infected with HTLV-I[22]. Sixteen percent of San Francisco IDU are HTLV seropositive, and most of these appear to be infected with HTLV-II[23]. A study of primarily white IDU from the Staten Island, New York area, found PCR-determined prevalences of eleven percent for HTLV-II and an additional nine percent for HTLV-I[24]. Finally, measurement of HTLV-I and -II antibodies in sera from the CDC-sponsored HIV sentinel counties survey yielded undifferentiated HTLV-I /II prevalences among IDU in methadone treatment centers ranging from 0.4% (Atlanta) to 17.6% (Los Angeles)[25]. Interestingly, there was little concordance in the ranking of cities by HIV prevalence versus HTLV-I/II prevalence.

Based upon the 2000 U.S. Census data, it is estimated that the total number of HTLV-II infected persons in the United States is approximately 197,000. This includes 56,000 in the general population (U.S. population 281,422,000 X 0.02% blood donor prevalence[8], 100,000 among IDU (1 million IDU X 10% prevalence[25]) and 41,000 among American Indians (4,119,000 Native American/Alaska natives X 1% prevalence[11]).

HTLV-I causes adult T-cell leukemia (ATL), a malignancy of mature CD4+ T-lymphocytes that presents most commonly as lymphoma with skin involvement and hypercalcemia.[26] HTLV-I is the causative agent of HAM**,** a slowly progressive spastic paraparesis that is characterized by weakness in the legs, diffuse hyperreflexia, clonus, loss of vibration sense, and detrusor insufficiency leading to bladder dysfunction. HTLV-I may also be associated with a wider spectrum of neurological manifestations that do not meet diagnostic criteria for HAM. Sensory neuropathy,[27-29] gait abnormalities,[30, 31] bladder

dysfunction,[27, 30-33] erectile dysfunction,[34, 35] amyotrophic lateral sclerosis (ALS),[36] mild cognitive deficits,[37] and rarely, motor neuropathies[27, 29, 34, 38-40] have all been reported among HTLV-I-infected individuals without HAM. HTLV-I infection has also been implicated in a spectrum of autoimmune conditions such as uveitis, arthritis, and pneumonitis, although there is good epidemiologic evidence of association only with uveitis and arthritis.[31, 41, 42]

Although HTLV-II was initially isolated from two patients with presumed hairy cell leukemia, it was subsequently determined that at least one of them had a dual disorder: HTLV-II negative B-cell hairy cell leukemia and HTLV-II positive CD8+ lymphoproliferative syndrome.[43] Cumulative evidence suggests that HTLV-II is associated with a myelopathic syndrome similar to HTLV-I related HAM. Including four cases from the HOST cohort, about a dozen cases of HTLV-II classical HAM have been diagnosed, including some with virologic evidence of HTLV-II in cerebrospinal fluid.[44-50] Araujo and Hall have recently reviewed the literature on HTLV-II myelopathy, and conclude that HTLV-II is in fact associated with rare cases of neurologic disease.[51]

b) Background and Rationale

Screening tests for HTLV-I/II are available, although the access to such assays is limited and intermittent. The U.S. blood banking industry employs a dual screening HTLV algorithm, which entails screening donor specimens by two licensed HTLV screening assays, in order to reduce the false positive results inherent in less specific screening assays, prior to additional supplemental testing required to discriminate antibodies to distinct viral antigens needed for donor notification and testing. Donor deferral occurs when the donor specimen is repeat reactive (RR) to HTLV-I/II antibodies based on a second licensed HTLV screening test of a different type[52]. Previously, the HTLV dual screening algorithm was employed using the Abbott HTLV-I/HTLV-II EIA and the Abbott PRISM HTLV-I/HTLV-II , but in 2009, Abbott discontinued the HTLV-I/HTLV-II EIA, creating a void of a second licensed screening assay. At present, there are now two licensed HTLV screening assays, the Abbott PRISM HTLV-I/HTLV-II chemiluminescent assay (CIA) and the AVIOQ HTLV-I/II Microelisa System; the AVIOQ HTLV-I/II Microelisa System was licensed in early 2012. However, there remains no licensed supplemental assay for confirmation and differentiation of viral antibodies.

RR specimens from HTLV screening tests require additional, more specific tests to confirm HTLV positivity, including discrimination of HTLV-I and HTLV-II. These supplemental assays can take many forms including specific peptides, enzyme-linked immunosorbent assays (ELISAs) or western blots. Supplemental enzyme immunoassays (EIA) based assays must be capable of identifying antibodies to core (GAG) and envelope (ENV) proteins of HTLV-I and HTLV-II; nucleic acid based assays must be capable of identifying specific pro-viral DNA sequences. A western blot assay with nitrocellulose strips incorporating recombinant antigens specific to HTLV-I and HTLV-II as well as HTLV-I native vital antigens is one such commonly used test. The MP Diagnostics HTLV Blot 2.4 has been used ex-U.S. as a

registered supplemental assay for the confirmation and discrimination of antibodies to HTLV-I and HTLV-II, and has been available in the U.S. as a research use only product.

Screening of whole blood donations for the presence of antibodies to HTLV-I/II has been required in the U.S. since 1988. The licensed HTLV screening assays available are sensitive and use of a dual screening algorithm reduces the incidence of false positives. Simple, yet specific and sensitive, supplemental serological tests are therefore needed to enable rapid confirmation and differentiation of HTLV-I and HTLV-II seropositive samples, which is recommended for donor counseling and notification.

Currently, there are no available licensed supplemental tests to confirm the presence of the antibody in a donor's sample. A supplemental test is essential to provide additional information to the donor for counseling, follow-up testing and / or treatment, and may be an option for donor re-entry in cases of a negative outcome. MP Biomedicals has submitted a Biologics License Application (BLA) for the MP Diagnostics HTLV Blot 2.4; if approved, the HTLV Blot 2.4 would be the first licensed confirmatory or supplemental assay available in the U.S.

c) Status of Product Development in HTLV

1) Research Use Only Products

In addition to the HTLV Blot 2.4, there are two (2) main products available for HTLV supplemental testing purposes. These products, available as research use only assays in the U.S., are the INNO-LIA™ HTLV I/II Score (Innogenetics, Belgium) and the California Department of Public Health Laboratories (CDPHL) HTLV supplemental testing algorithm (State Laboratory, California), which has been used by the ARC since 2002.

The INNO-LIA™ HTLV I/II Score is a line immunoassay (LIA) that confirms HTLV infection and differentiates between HTLV types 1 and 2 through the use of recombinant antigens and synthetic peptides. Non-type specific antigens, two *gag* proteins (i.e. p19 I/II, p24 I/II) and two *env* proteins (i.e. gp46 I/II, gp21 I/II), are used to confirm the presence of HTLV antibodies in a specimen. Type specific antigens, *gag* p19-I, type specific for HTLV-I, gp46-I, type specific for HTLV-I, and gp46-II, type specific for HTLV-II, are used to differentiate between infection type. In contrast to the HTLV Blot 2.4, the HTLV I/II Score does not use a native viral lysate, but instead requires on the presence of a recombinant gp46 derived antigen for HTLV type differentiation. A clinical study is listed as beginning July of 2013 on National Institutes of Health (NIH) website ClinicalTrials.gov; currently however, the HTLV I/II Score is available only as Research Use Only (RUO) in the U.S.

The CDPHL HTLV Supplemental Testing Algorithm consists of a series of laboratory developed tests (LDT) including an in-house HTLV ELISA, an indirect fluorescent antibody assay (IFA), a Western blot, and a radio immunoprecipitation assay (RIPA), run in a defined sequence. The overall interpretation is determined by the results of all tests performed as indicated in ***Figure 01.***

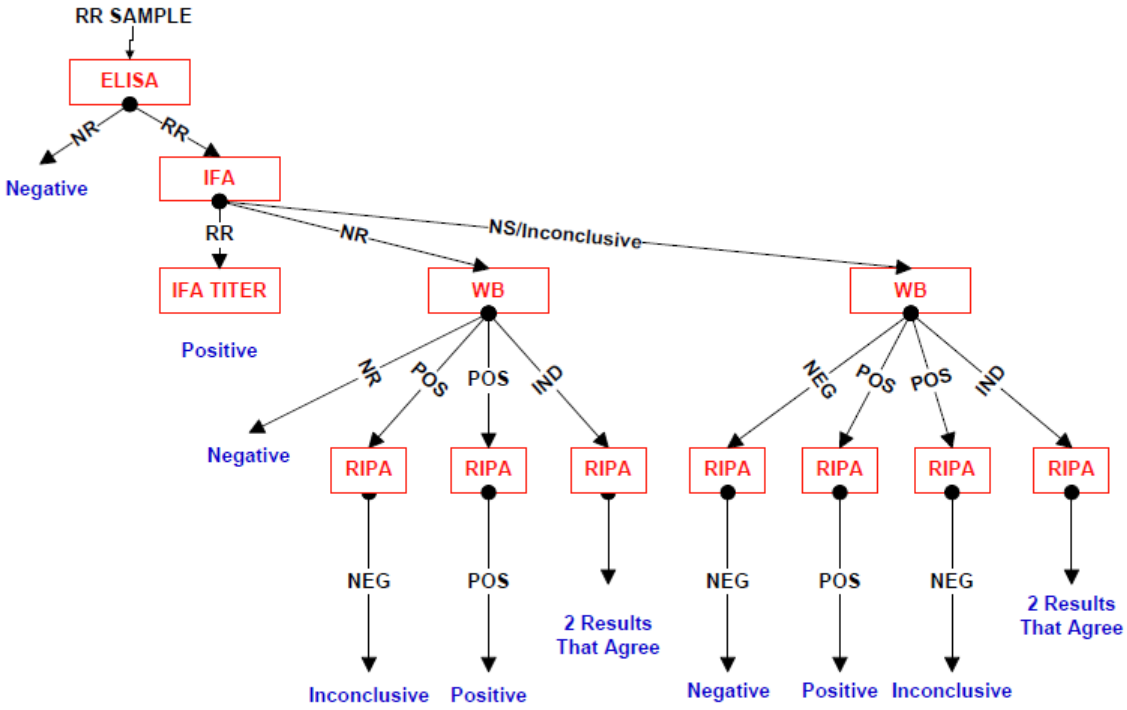


Figure 01: CDPHL HTLV Supplemental Testing Algorithm

Samples that are RR on the CDPHL HTLV ELISA, defined as two out of three tests above the assay cut-off (COV), are subjected to further testing by an HTLV IFA. If the HTLV IFA is reactive to both HTLV-I and HTLV-II antigens, the sample is typed using IFA endpoint titration to determine if the sample is HTLV-I or HTLV-II, and the sample is reported as antibody detected with the HTLV sub-type differentiated. A reaction to one antigen and not the other is considered to be inconclusive; samples non-reactive or inconclusive on IFA proceed to Western blot testing.

The CDPHL HTLV Western blot assay is an EIA that utilizes a combination of native proteins derived from a HTLV-I viral lysate along with a recombinant p21e antigen. This is similar to the HTLV Blot 2.4, which uses a combination of native viral proteins from a HTLV-I viral lysate along with not one, but three recombinant antigens: rgp46-I (MTA-1); rgp46-II (K-55); and GD21. Both assays are based on the principle that antibodies to HTLV-I and HTLV-II, if present in the donor's samples, will bind to the antigens immobilized in nitrocellulose strips. The interpretation of the CDPHL western blot is based on

the presence or absence of six bands: p19; p24; p28; p36; rg46; p53; and p21e. A positive sample on the CDPHL HTLV western blot will show the presence of the p19 and / or p24 bands, plus p21e. Samples are indeterminate if p21e only is present, and negative if p21e is absent regardless of the presence of core bands p19 and/or p24. If two of the test results are concordant, that result will be reported out as the overall interpretation (i.e. antibody detected or not detected); samples with discordant IFA and western blot results will be tested using an in-house RIPA.

The in-house RIPA is performed in cases where the sample IFA and western blot results are discordant, or when a sample has a weak antibody response with a pattern of EIA reactive, IFA non-reactive and Western Blot positive. The overall interpretation is antibody positive, but not typed, when the RIPA is positive and inconclusive when the RIPA is negative.

2) Product Comparison

The HTLV Blot 2.4, the HTLV I/II Score, and the CDPHL HTLV Western blot are all EIA based assays that share many product design similarities. Additionally, they share similarly based interpretation criterion, especially in regards to a single or combination of HTLV *gag* proteins considered seronegative. **Reference Section 9c: HTLV Gag Indeterminate Profile / Gag Indeterminate Profiles** for the discussion on the significance of *gag* proteins in HTLV interpretation criteria. An outline of the similarities and differences between the three products are indicated in **Table 04.**

Table 04: HTLV Supplemental Assay (Research Use Only) Comparison Table

Product	HTLV Blot 2.4 (MP Biomedicals Asia Pacific)	HTLV I/II Score (Innogenetics, Belgium)	HTLV Western Blot (State of CA)
Components	Recombinant antigens (rgp46-I, rgp46-II, GD21) and native viral lysate proteins (p19, p24, etc)	Recombinant proteins and synthetic peptides (p19 I/II, p24 I/II, gp46 I/II, gp21 I/II, p19-I, gp46-I, gp46-II)	Viral lysate (p19, p24, p28, p36, gp46, p53) and recombinant antigen p21e
Intended Use	Confirmation and differentiation	Confirmation, followed by differentiation	Confirmation only
Confirmation Bands	GD21, p19, p24, rgp46-II, rgp46-I	p19 I/II, p24 I/II, gp46 I/II, gp21 I/II	p21e, and p19 and/or p24
Differentiation Bands	Same as above	p19-I, gp46-I, gp46-II	N/A
Negative Criteria	No HTLV bands Single *gag* band or any combination of *gag* bands, excluding p24 HGIP patterns	No HTLV bands Single band (p19 I/II, p24 I/II, gp46 I/II)	Absence of p21e is negative, regardless of presence of p19 and/or p24
Indeterminate Criteria	Any non-specific pattern	gp21 I/II alone two bands present with gp21 I/II non-reactive	p21e only present
HTLV-I Criteria	GD21, p19 and rgp46-I GD21, p19, p24, with p19 ≥ p24	p19-I, gp46-I	p19 and/or p24, with p21e
HTLV-II Criteria	GD21, p24, rgp46-II GD21, p19, p24, with p24 > p19	gp46-II	N/A
HTLV-I/II	GD21, p19, p24, rgp46-II, rgp46-I	N/A	N/A

7. DEVELOPMENT OF THE MP DIAGNOSTICS HTLV BLOT 2.4

a) MP Biomedicals Company Overview

MP Biomedicals, LLC is a worldwide corporation headquartered in Southern California, with ISO-certified and FDA-approved manufacturing and distribution facilities throughout the globe. MP Biomedicals Asia Pacific Pte Ltd, a subsidiary of MP Biomedicals, LLC based in Singapore, is the manufacturer of the MP Diagnostics HTLV Blot 2.4.

The history of MP Biomedicals Asia Pacific Pte Ltd began in 1985 with the formation of Diagnostics Biotechnology (DB), an in vitro diagnostic (IVD) company focused on product development for infectious disease, including Human Immunodeficiency Virus (HIV) and HTLV. In 1993, U.S. based company Genelabs Technologies, Inc (GLT) acquired DB and formed a Singapore subsidiary, Genelabs Diagnostics Pte Ltd (GLD). GLD remained focused on the development and manufacture of IVD devices for the detection of infectious diseases, and expanded the original DB product offering to include additional screening and confirmatory assays for various diseases. In 2004, MP Biomedicals, LLC acquired GLD, forming the company known today as MP Biomedicals Asia Pacific Pte Ltd. The focus of MP Biomedicals Asia Pacific Pte Ltd remains as the development, manufacture, and marketing of IVD products for the global diagnostics market. Initially focused on registrations in European and Asian markets, MP Biomedicals Asia Pacific is now looking towards the U.S. market in order to meet previously unmet needs in small, niche infectious disease markets such as HTLV. The MP Diagnostics HTLV Blot 2.4 BLA is the company's first submission for the U.S. regulatory market in history.

MP Biomedicals, LLC, and all its subsidiaries, qualifies and is certified as a Small Business by the Food and Drug Administration (FDA) under the Medical Device User Fee Act (MDUFA).

b) Product Development Program

The HTLV Blot has been available in different iterations available on the global market since 1985. Under the DB company name, the HTLV Blot 1.2 was first introduced containing HTLV-I viral lysate in MT-2 cells, allowing for confirmation of HTLV-I only. In 1987, the HTLV-I Blot 2.0, which included recombinant antigen p21e, a derivative of gene product gp21, was developed and introduced, and allowed for further confirmation of HTLV-I infection. Addition of recombinant antigen MTA-4 in 1989 to the existing blot produced iteration HTLV Blot 2.2, and allowed for further confirmation of HTLV-I infection using two (2) recombinant antigens for HTLV-I. A subsequent iteration of the product, the HTLV Blot 2.3, was developed in 1991. In the HTLV Blot 2.3, MTA-1, an epitope that demonstrated an increased immunoreactivity to diverse populations of HTLV-I, replaced MTA-4, increasing sensitivity. K-55, an

HTLV-II type specific epitope was added along with anti-human IgG control band, and an HTLV-I viral lysate in HuT102 cells replaced the existing HTLV-I viral lysate in MT-2 cells.

In 1993, the final iteration of the HTLV Blot, the HTLV Blot 2.4, was introduced under the company GLD. This iteration replaced recombinant antigen p21e with GD21, an epitope derived from a truncated portion of the p21e gene product and which demonstrated a more specific immunoreactivity to both HTLV-I and HTLV-II; p21e had previously demonstrated non-specific immunoreactivity with sera from individuals shown to be uninfected with HTLV-I or HTLV-II. The HTLV Blot 2.4 was sold globally generally as a research use only product until its first product registration in 1995 under the company name GLD, and again in 1999 when it was first registered as a Class IV product with Health Canada. These initial registrations of the HTLV Blot 2.4 were soon followed by the well-recognized CE mark in 2004, which included a revised interpretation criteria defining HTLV *gag* indeterminate profiles (HGIP), as well as single and combination *gag* indeterminate profiles without p24 present, as seronegative. Subsequent country registrations followed as indicated in **Table 05.** Currently, the HTLV Blot 2.4 is available in more than 26 countries as an HTLV supplemental assay, intended for HTLV confirmation and type differentiation for both donor and patient samples. . More than 780,000 tests of HTLV Blot 2.4 were sold globally since 1995

Table 05: HTLV Blot 2.4 Product Registrations.

Country	Registration Year
Taiwan	1995
Canada	1999
European Union	2004
Singapore	2005
Columbia	2008
Brazil	2011
India	2011
Ecuador	2012
Panama	2012

c) U.S. Regulatory History

As mentioned previously, the blood banking industry employs a dual screening algorithm for HTLV, which means that donor specimens are screened by two different licensed HTLV screening assays. Only those specimens that are dual reactive, or repeat reactive by both licensed assays, will progress to supplemental testing. In 2009, Abbott discontinued their HTLV-I/HTLV-II EIA, leaving the industry with only one licensed HTLV screening assay, the Abbott PRISM HTLV-I/HTLV-II. This discontinuation left the industry with an unmet need for a second licensed HTLV screening assay, and actions were taken to fill this gap.

MP Biomedicals currently offers an HTLV screening assay, the MP Diagnostics HTLV-I/II ELISA 4.0, as a research use only assay available in the U.S. Upon discontinuation of the HTLV-I/HTLV-II EIA, MP Biomedicals was contacted by Jerry Holmberg, Senior Advisor for Blood Policy, FDA, regarding their interest in submitting an Investigational New Drug (IND) application for HTLV-I/II for blood, organ and tissue screening. Subsequent communication ensued, resulting in a pre-IND meeting in December of 2009. During the pre-IND meeting, MP Biomedicals was asked about their interest in seeking licensure of the MP Diagnostics HTLV Blot 2.4 as a supplemental test. Midyear in 2010, MP Biomedicals submitted a bundled IND that included the HTLV-I/II ELISA 4.0, intended use as a screening assay, and the HTLV Blot 2.4, intended use as a supplemental assay. Activities on the IND continued until midyear 2011 when, MP Biomedicals split the studies, placing the HTLV-I/II ELISA 4.0 project on hold and continuing with the smaller HTLV Blot 2.4 study. Clinical trials for the HTLV Blot 2.4 were initiated at the end of 2011, continuing to July of 2012. In early 2013, MP Biomedicals submitted the first BLA for the company, which was accepted in May of 2013 and assigned an action due date of January 4, 2014.

8. PRODUCT INFORMATION

a) Intended Use

The MP Diagnostics HTLV Blot 2.4 is a qualitative enzyme immunoassay intended for confirming and differentiating the presence of antibodies to HTLV-I and HTLV-II in human serum or plasma. It is intended for use as a more specific supplemental test for human serum or plasma samples with repeatedly reactive results by an FDA-approved HTLV I/II screening test.

The MP Diagnostics HTLV Blot 2.4 is intended for use in a manual mode or a semi-automated mode using the MP Diagnostics AutoBlot System 20.

b) Assay Principle

The MP Diagnostics™ HTLV Blot 2.4 is a qualitative EIA that uses well-defined antigens derived from HTLV-I and HTLV-II viral and recombinant proteins **(Reference Section 8e: HTLV Antigen Selection)**. The HTLV-I viral proteins are derived from native inactivated disrupted viral particles and genetically engineered proteins, then highly purified and fixed on a nylon membrane (nitrocellulose strips). The sequences are selected to allow the detection of antibodies with a wide specificity to all known isolates of the HTLV strains. The antigenicity exhibited by these proteins is either common to HTLV-I and HTLV-II antibodies or type-specific to one of the two viruses to allow confirmation and discrimination in a single assay. This is accomplished by incorporating MTA-1, a unique HTLV-I envelope recombinant protein (rgp46-l), K55, a unique HTLV-II envelope recombinant protein (rgp46-II) and GD21, a common yet specific HTLV-I and HTLV-II epitope envelope recombinant protein (rgp21). Each strip also includes an internal sample addition control to minimize the risk of false negatives due to operational errors.

Individual nitrocellulose strips are incubated for one hour in a test trough with diluted serum or plasma specimens and controls. Specific antibodies to HTLV-I/II, if present in the specimen, will bind to the HTLV-I/II proteins on the strips. The strips are washed to remove unbound materials. Antibodies that bind specifically to the HTLV proteins can be visualized after incubating with goat anti-human IgG conjugated with alkaline phosphatase and washing, then incubating with the substrate, BCIP/NBT. This substrate produces a dark color as bands on the strip in proportion to the amount of specific antibodies present in the sample. The color development is stopped with reagent grade water. If the sample contains no HTLV-specific antibodies, only a low background color develops with a visual serum control band. This method is sensitive enough to detect marginal amounts of HTLV antibodies in serum or plasma.

The bands are read visually and reported as seronegative, indeterminate, HTLV-I seropositive, HTLV-II seropositive, or HTLV-I/II seropositive based on the band(s) present compared to a control strip. Additionally, identification of specific protein bands is assisted through the use of a lot specific "Protein Finder" ***(Figure 02)***. Grading of individual band intensity by scores such as +1, +2, +3, is facilitated through the use of a "Intensity Finder" provided ***(Figure 03)***. Finally, organization, storage and scoring of the nitrocellulose strips are facilitated through the use of a "Report Sheet", provided with every kit ***(Figure 04)***.

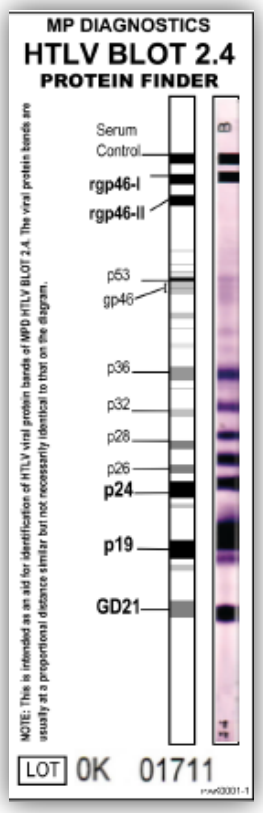


Figure 02: HTLV Blot 2.4 Protein Finder

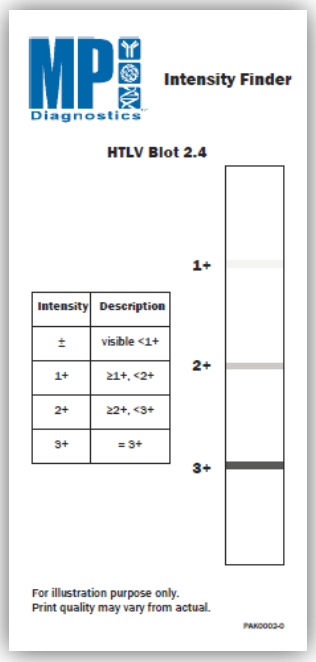
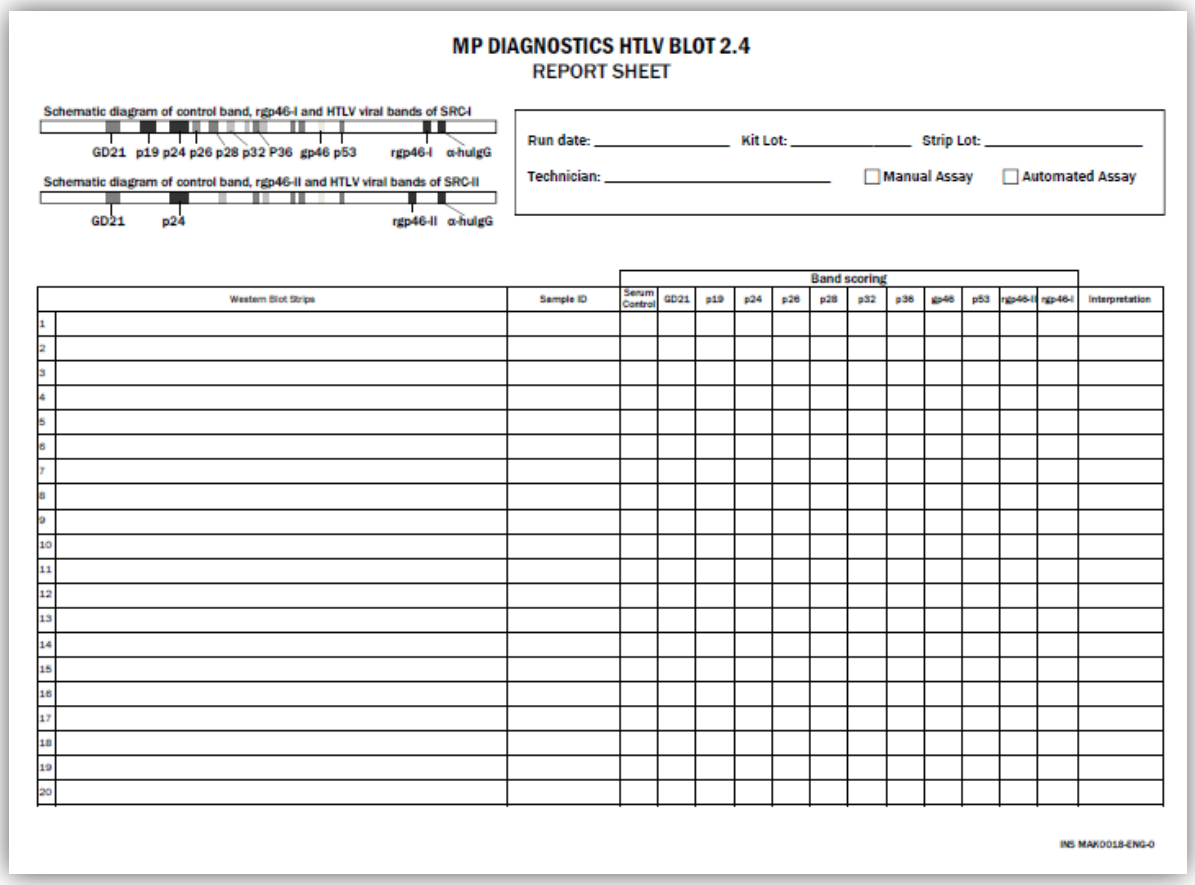


Figure 03: HTLV Blot 2.4 Intensity Finder



MP DIAGNOSTICS HTLV BLOT 2.4
REPORT SHEET

Schematic diagram of control band, rgp46-I and HTLV viral bands of SRC-I

GD21 p19 p24 p26 p28 p32 P36 gp46 p53 rgp46-I α-hulgG

Schematic diagram of control band, rgp46-II and HTLV viral bands of SRC-II

GD21 p24 rgp46-II α-hulgG

Run date: _____ Kit Lot: _____ Strip Lot: _____

Technician: _____ ☐ Manual Assay ☐ Automated Assay

	Western Blot Strips	Sample ID	Band scoring: Serum Control	GD21	p19	p24	p26	p28	p32	p36	gp46	p53	rgp46-II	rgp46-I	Interpretation
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19															
20															

INS MAK0018-ENG-0

Figure 04: HTLV Blot 2.4 Report Sheet

c) Reagents and Materials Provided

The MP Diagnostics HTLV Blot 2.4 kit is designed to meet the individual needs of diverse end users, and is therefore available in different kit size configurations that contain the components as listed in **Table 06** below. The HTLV Blot 2.4 kit is available in test kit sizes of 18 tests or 36 tests; the quantities of components for each size configuration are indicated in **Table 07.** The HTLV Blot 2.4 is designed to be run manually or using the MP Diagnostics AutoBlot System 20, and there is no difference in the kit configuration for either manual or automated use. Additionally, the final to-be-marketed HTLV Blot 2.4 formulation is equivalent to that of the Investigational Use Only (IUO) product used in the U.S. clinical trials, as well as to that of the CE mark version registered in 2004.

Table 06: HTLV Blot 2.4 Kit Components

Component Name	Description
Nitrocellulose Strips	Incorporated with recombinant antigens (MTA-1, K55, GD21), HTLV-I viral lysate, and anti-human IgG band.
Non-Reactive Control	Inactivated normal human serum with documented non-reactivity for anti-HCV, anti-HIV-1/2, anti-HTLV-I/II, and HBsAg.
HTLV Strong Reactive Control I	Inactivated human serum with high tittered antibodies to HTLV-I and non-reactive for anti-HCV, anti-HIV-1/2 and HBsAg.
HTLV Strong Reactive Control II	Inactivated human serum with high tittered antibodies to HTLV-II and non-reactive for anti-HCV, anti-HIV-1/2 and HBsAg.
Lyophilized Stock Buffer	Tris buffer with heat inactivated animal and non-animal proteins
Wash Buffer Concentrate (20X)	Tris with Tween – 20
Conjugate	Goat anti-human IgG conjugated with alkaline phosphatase.
Substrate	Solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT)
Blotting Powder	Non-fat dry milk
Instructions for Use	Written directions for product use
Forceps	Tool to handle strips
Protein Finder	Lot specific nitrocellulose strip with HTLV viral bands identified
Intensity Finder	Intensity scale for identifying weak samples or relative intensities
Record Sheet	Sheet for organizing, storing and interpreting nitrocellulose strips

Table 07: HTLV Blot 2.4 Kit Component Quantity Per Kit Size

Component Name	Quantity Per 18 Test Kit	Quantity Per 36 Test Kit
Nitrocellulose Strips	18 strips	36 strips
Non-Reactive Control	1 x 80 µL	1 x 80 µL
HTLV Strong Reactive Control I	1 x 80 µL	1 x 80 µL
HTLV Strong Reactive Control II	1 x 80 µL	1 x 80 µL
Lyophilized Stock Buffer	1 bottle reconstituted to 100 mL	2 bottles reconstituted to 100 mL each
Wash Buffer Concentrate (20X)	1 x 70 mL	1 x 70 mL
Conjugate	1 x 160 µL	1 x 160 µL
Substrate	1 x 100 mL	1 x 100 mL
Blotting Powder	10 x 1 g packets	10 x 1 g packets

d) Modes of Operation

As previously indicated, the HTLV Blot 2.4 is designed to be performed manually, using standard, commercially available, laboratory equipment, or automated, using the MP Diagnostics AutoBlot System 20 ***(Figure 05)***. The AutoBlot is a firmware-controlled device that automates the processing steps of the HTLV Blot 2.4. The processing steps that may be performed include dispensing of reagents, aspiration and incubation of the nitrocellulose strips after sample application. The Nitrocellulose Strips, as well as the specimens and controls, are added manually, and all strips are manually resulted. The instrument is capable of dispensing reagents for, and aspirating up to, 20 strips in 90 seconds; it is programmed to dispense up to five reagents with one pump included per reagent.

Figure 05: MP Diagnostics AutoBlot System 20

The functionality of the AutoBlot is limited to the dispensing and aspirating of reagents into and out of an AutoBlot tray respectively, incubation of the tray at appropriate times in the assay, rocking of the tray using a motorized platform, and storage of the HTLV Blot 2.4 assay program only.

Following the manual addition of test samples, the AutoBlot System 20 incubates, washes, and performs subsequent reagent additions as defined by the assay program. It permits easy setup with walk-away performance, sounding an alarm when the test is complete. The functionality does not extend to detection or measurement of HTLV bands on the nitrocellulose strips, nor storage of assay results.

The MP Diagnostics™ AutoBlot System 20 is automated by firmware that controls the dispense / aspirate arm. The customized MP Diagnostics™ HTLV Blot 2.4 assay is preprogrammed into the AutoBlot; no other programmable assays are allowed. The firmware automates the addition of reagents, as well as notifies the operator to manually add the nitrocellulose strips, and samples and controls. The AutoBlot then proceeds to cycle through the programmed steps of the assay until completion of processing.

Each assay run requires that positive and negative controls are placed in the troughs farthest from the aspirate arm drip tray, with the negative control furthest from the tray. This ensures that any problems with incorrect arm movement, loss of reagent, or cross contamination is evident in the controls.

Reading and interpretation of the strips are performed manually.The AutoBlot complies with the essential requirements of the applicable European laws and Directives 98/79/EC, under Annex III with respect to safety, health, environment and consumer protection and has been listed since July 2, 2004. The AutoBlot carries the MET mark, meaning that it has been evaluated and certified by MET Laboratories, Inc. in meeting the requirements of: UL61010-1/CSA C22.2 No. 61010-1, 2nd Edition: Standard for Laboratory Equipment, Rev. July 12, 2004; UL61010-2-10, 1st Edition: Particular Standard for Heating Equipment, Rev. March 13, 2002; EN 61326-1: 1998 wit EN 55011 (CISPR 11): 1998; and FCC Verification Rules Contained in Title 47 of the CFR, Part 15, Subpart B For a Class A Digital Device.

e) HTLV Antigen Selection

As previously mentioned, the HTLV Blot went through several iterations before its final configuration as commercially available HTLV Blot 2.4. The majority of changes included improvements to the selection of HTLV antigens used in the confirmation and differentiation of HTLV type 1 and type 2 infection. A complete discussion of the final HTLV interpretation criteria utilized by the HTLV Blot 2.4 is included in **Section 9a: Overview of HTLV Blot 2.4 Criteria.** In general, the HTLV Blot 2.4 uses the presence or absence of five (5) key HTLV proteins, GD21, rgp46-I (MTA-1), rgp46-II (K-55), p19, and p24 for confirmation and discrimination. Additionally, the HTLV Blot 2.4 includes an anti-human IgG protein to ensure assay validity and sample addition.

1) GD21

GD21 is a recombinant transmembrane protein derived from the human T-cell lymphotropic virus (HTLV). Specifically, it derives from a highly conserved region of the HTLV-I gp21 envelope protein sequence, antigenic region 6261 – 6392 ***(Figure 06)***. It is intended for use in the production of Western Blot and Enzyme-Linked Immunosorbent Assays (ELISA) for detection to antibodies to HTLV-I/II in human plasma or serum.

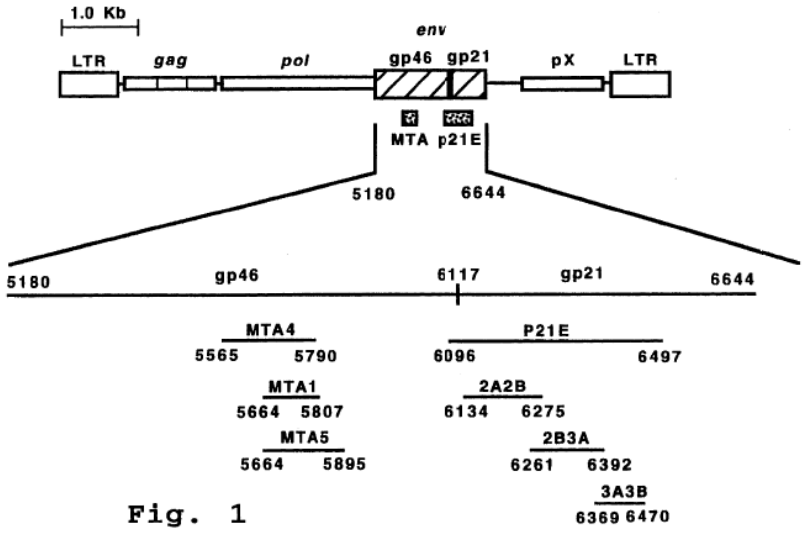


Figure 06: Derivation of Recombinant GD21 (2B3A)[a]

[a] Source: U.S. Patent 5, 643,714 Figure 1

The GD21 protein is produced by cloning the sequence into a modified *Escherichia coli (E. Coli)* pGEX vector containing a truncated Gluthathione S-Transferase (GST) sequence (pGEX.del65) using the restriction sites NcoI and BamHI to create a pGEX.GD21.del65 plasmid. The plasmids are then transformed into Rosetta™ 2 (DE3) Competent Cells, a commercially available cell line from EMD Millipore. GD21 clones are ampicilin and chloramphenicol resistant.

Following culturing of the GD21 clones, nickel affinity and size exclusion chromatography is used to purify the protein, which in its final form is a clear liquid in an 8M Urea, 10 mM Tris, 100 mM NaH_2PO_4, solution of pH 8.0. GD21 exhibits strong reactivity to HTLV-I and HTLV-II sera but is non-reactive with HTLV-I/HTLV-II negative sera as shown by Western blotting and manual slotting. Specifically, the GD21 protein has non-immunoreactivity with HTLV-I/II negative sera that has been shown to be immunoreactive to the p21e antigen. The product is miscible in water; greater than 95% pure; has a protein concentration between 1.0 – 3.0 mg/ml and; a shelf-life of 12 months at -80^{o} C.

GD21 is a 16k dalton recombinant protein as determined by SDS-PAGE and amino acid composition obtained by translation of its DNA construct. As identified in U.S. Patent Number 5,643,714, “Method and Assay for HTLV”, dated July 01, 1997, GD21is a linear protein, 44 amino acids in length, with the sequence description as indicated in ***Figure 07.***

Ile Val Lys Asn His Xaa Asn Xaa Leu Xaa Xaa Ala Gln Tyr Ala Ala Gln Asn Arg Arg Gly Leu

1 5 10 15 20

Asp Leu Leu Phe Trp Glu Gln Gly Gly Leu Cys Lys Ala Xaa Gln Glu Gln Cys Xaa Phe Xaa Asn

25 30 35 40

Figure 07: GD21 Amino Acid Sequence

Patent U.S. 5, 643,714 details the analyses used to demonstrate the activity of the GD21 protein, immunoreactive to sera from human subjects infected with HTLV-I or HTLV-II and non-immunoreactive to sera that is immunoreactive with p21e antigen but obtained from human subjects not infected with HTLV-I or HTLV-II[53].

Immunoreactivity of the GD21 protein, or 2B3A peptide, was evaluated through preparation of bacterial lysates and screening of these lysates with sera of known reactivity. GD21 expression vectors were constructed by amplifying a fully-copy HTLV-I genome DNA insert using site-specific primers, and then ligating into a modified pGEX-GLI vector for transformation of E. Coli host cells. Lysates prepared from cultures of the transformed E. Coli were loaded and electrophoresed on a 12% polyacrylamide SDS gel, then blotted onto nitrocellulose paper. The resulting Western blots were incubated overnight in the presence of human sera with known HTLV status, and examined for immunoreactivity. The activity of GD21, or 2B3A peptide, is indicated in **Table 08.**

Table 08: Reactivity of GD21 (2B3A) Recombinant[a]

Sera	Type	p21E	1A1B	2A3B	2A2B	2B3A	3A3B
J254	I	+	+	+	-	+	-
J253	I	+	-	+	-	+	-
J183	I	+	ND	+	-	+	-
J313	I	+	-	+	-	+	-
J103	I	+	-	+	-	+	+
J332	II	+	-	+	-	+	-
J317	II	+	-	+	-	+	+
J309	II	+	-	+	-	+	-
GE9	Sup	+	-	+	-	+	-
5E4	Sup	+	-	+	-	+	-
J376	Uninf	-	-	-	-	-	-
JCO1	Ind	+	ND	+	-	-	+
JC02	Ind	+	ND	+	-	-	+
JCO3	Ind	+	ND	+	-	-	+
JCO4	Ind	+	ND	+	-	-	+
JCO5	Ind	+	ND	+	-	-	+
JCO6	Ind	+	ND	+	-	-	+
JCO7	Ind	+	ND	+	-	-	+

[a]U.S. Patent 5, 643,714 – Table 1

Legend: ND – not done; I – HTLV-I; II – HTLV-II; UnInf – uninfected individual; Sup – tissue culture supernatant; Ind – sera that are reactive with the recombinant protein p21E but that are negative for the presence of HTLV-I or HTLV-II infection by PCR

As depicted in **Table 08,** isolate 2B3A, or GD21, was non-reactive with the PCR negative samples previously showing reactivity to p21e (JC01 – JC07). Additionally, GD21 has demonstrated no cross immunoreactivity to sera from individuals infected with HIV, Hepatitis B virus (HBV), or Hepatitis C virus (HCV). All stated immunoreactivity has been confirmed through extensive in-house testing and qualification performed at MP Biomedicals Asia Pacific Pte Ltd during assay verification and validation activities. Subsequent production analyses support the activity of the GD21 protein as immunoreactive to HTLV-I and HTLV-II, with demonstrated specificity in the presence of potentially cross-reactive *in vitro* substances, non-immunoreactivity to sera from individual uninfected with HTLV-I/II, and non-immunoreactivity to sera from individuals immunoreactive to the p21e antigen and PCR negative.

2) rgp46-I (MTA-1$^{162-209}$)

MTA-1 is a recombinant protein derived from a variable region of the human T-cell lymphotropic virus I (HTLV-I) gp46 envelope protein sequence cloned into *Escherichia coli (E. Coli)* pGEX. Specifically, it derives from a highly conserved region of the HTLV-I gp46 envelope protein sequence, antigenic region 5664-5807 ***(Figure 08)***. It is intended for use in the production of Western Blot and Enzyme-Linked Immunosorbent Assays (ELISA) for detection to antibodies to HTLV in human plasma or serum.

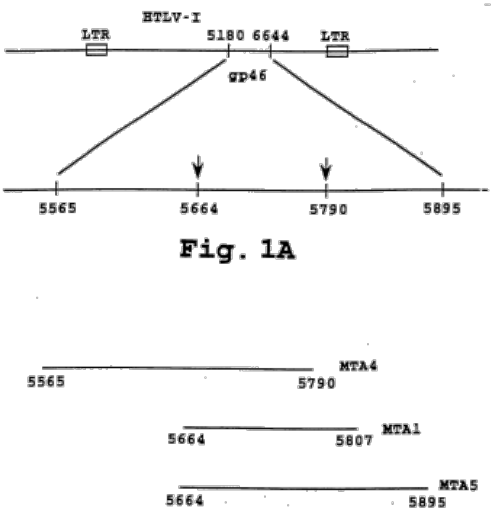


Figure 08: rgp46-I (MTA-1) Antigenic Derivation[a]

[a] Source: U.S. Patent 5,088,579 Figure 1A & 1B

MTA-1 is a 32 kDa recombinant protein as determined by SDS-PAGE and amino acid composition obtained by translation of its DNA construct. As identified in United States (U.S.) Patent Number 6,110,662, "HTLV-I/HTLV Assay and Method", dated August 29, 2000, MTA-1 is a linear protein, 48 amino acids in length, with the sequence description as indicated in ***Figure 09.***

```
Ser Leu Leu Val Asp Ala Pro Gly Tyr Asp Pro Ile Trp Phe Leu Asn
1               5                   10                  15

Thr Glu Pro Ser Gln Leu Pro Pro Thr Ala Pro Pro Leu Leu Pro His
            20                  25                  30

Ser Asn Leu Asp His Ile Leu Glu Pro Ser Ile Pro Trp Lys Ser Lys
        35                  40                  45
```

Figure 09: MTA-1 Amino Acid Sequence Description[a]

[a] Source: U.S. Patent 6,110,662

The MTA-1 protein is produced by cloning the sequence into a pGEX vector containing a full Gluthathione S-Transferase (GST) sequence (pGEX.GST). The MTA-1 deoxyribonucleic acid (DNA) insert is cloned using restriction enzyme sites EcoR1 and BamHI to create a pGEX.MTA-1 plasmid. The

plasmids are then transformed into Rosetta™ 2 (DE3) Competent Cells, a commercially available cell line from EMD Millipore. MTA-1 clones are ampicillin and chloramphenicol resistant.

The MTA-1 recombinant protein is purified by GST affinity and size-exclusion chromatography and in its final form is a clear colorless liquid in 6M Urea, 10 mM DTT, 1x PBS, solution at pH 7.5. MTA-1 exhibits strong reactivity with HTLV-I positive sera. It is non-reactive to HTLV-II positive sera and non-infected sera as shown by Western blotting and manual slot blot. The product is greater than 95% pure, has a protein concentration between 1.0 – 3.0 mg/ml and a shelf life of 16 months from the date of manufacture if stored at -80^{o} C. Repeat freeze-thawing should be avoided.

MTA-1 is a recombinant protein analogous to K55, however MTA-1 was cloned from the HTLV-I genome whereas K55 was cloned from HTLV-II[54]. When the two recombinant proteins are immobilized to nitrocellulose for Western blotting, the MTA-1protein reacts with HTLV-1 sera but not HTLV-II sera or sera from non-infected individuals as indicated in ***Figure 10.*** HTLV infections were verified by PCR analysis of blood cells.

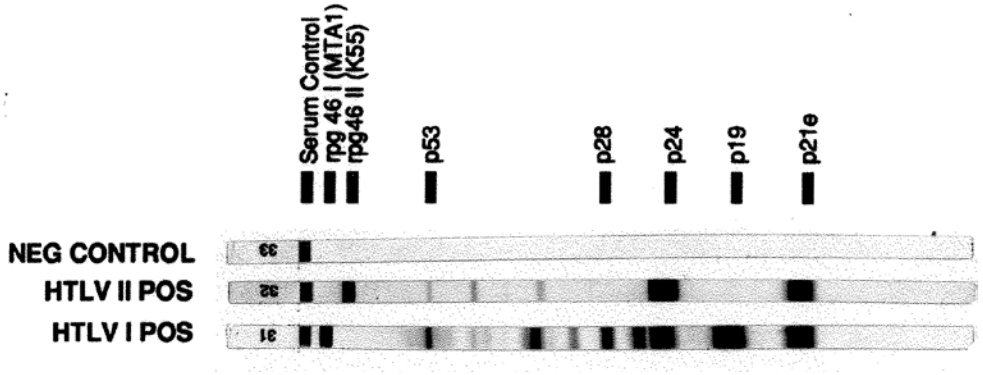


Figure 10: Western blot demonstrating MTA-1 specificity to HTLV-I[a]

[a] Source: AABB 1993 Meeting Abstract

Additionally, MTA-1 has demonstrated no cross immunoreactivity to sera from individuals infected with HIV, HBV, or HCV. All stated immunoreactivity has been confirmed through extensive in-house testing and qualification performed at MP Biomedicals Asia Pacific Pte Ltd during antigen verification and validation activities. Subsequent production analyses support the activity of the MTA-1 protein as immunoreactive to HTLV-I and non-immunoreactive to HTLV-II, and with demonstrated specificity in the presence of potentially cross-reactive *in vitro* substances.

3) rgp46-II (K-55$^{162-205)}$

HTLV type 1 and type 2 share at least 65% nucleic acid homology and 70% amino acid homology[54]. K55, a fusion protein derived from an envelope protein on HTLV-II, is a component of the HTLV Blot 2.4 and as shown below exhibits immunoreactive specificity to sera from patients infected with HTLV-II (non-reactive to sera from uninfected patients or those infected with HTLV-I).

K55 is a recombinant cell surface protein derived from a variable region of the human T-cell lymphotropic virus, type 2 (HTLV-II). Specifically, it derives from a highly conserved region of the HTLV-II gp46 envelope protein sequence, antigenic region 5663- 5794 ***(Figure 11)***. It is intended for use in the production of Western Blot and Enzyme-Linked Immunosorbent Assays (ELISA) for detection to antibodies to HTLV-II in human plasma or serum.

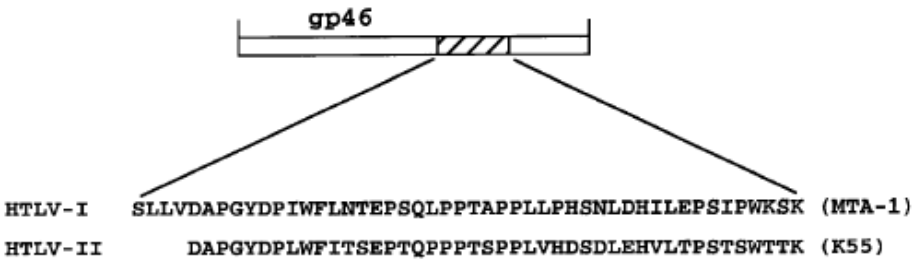


Fig. 2C

Figure 11: rgp46-II (K-55) Derivation[a]

[a] Source: U.S. Patent 5,088,579 Figure 2C

The K55 protein is produced by cloning the sequence into a modified *Escherichia coli (E. Coli)* pGEX vector containing a full Glutathione S-Transferase (GST) sequence (pGEX-GL1) using the restriction sites NcoI and BamHI to create a pGEX-GL1.K55 plasmid. The plasmids are then transformed into a version of *E. coli* cells. K55 clones are ampicillin and kanamycin resistant.

Following culturing of the K55 clones, Glutathione Sepharose 4B and size exclusion chromatography is used to purify the protein, which in its final form is a clear liquid in a 6M Urea, 10 mM DTT, 10 mM EDTA, 1x MTPBS, solution of pH 8.0. K55 exhibits strong reactivity and specificity to HTLV-II sera. It is non-reactive to HTLV-1 positive sera and non-infected sera as shown by Western blotting and manual slot blot. The product is greater than 95% pure, has a protein concentration between 1.0 – 3.0 mg/ml and

a shelf life of 23 months years from the date of manufacture if stored at -80° C. Repeat freeze-thawing should be avoided.

K55 is a 31 kDa recombinant protein as determined by SDS-PAGE and amino acid composition obtained by translation of its DNA construct. As identified in United States (U.S.) Patent Number 6,110,662, "HTLV-I/HTLV Assay and Method", dated August 29, 2000**,** K55 is a linear protein, 44 amino acids in length, with the sequence description as indicated in ***Figure 12.***

```
Asp Ala Pro Gly Tyr Asp Pro Leu Trp Phe Ile Thr Ser Glu Pro Thr
1               5                   10                  15

Gln Pro Pro Pro Thr Ser Pro Pro Leu Val His Asp Ser Asp Leu Glu
            20                  25                  30

His Val Leu Thr Pro Ser Thr Ser Trp Thr Thr Lys
        35                  40
```

Figure 12: K55 Amino Acid Sequence Description[a]

[a] Source: U.S. Patent 6,110,662 No. 5

As mentioned earlier, K-55 is a recombinant protein analogous to MTA-1, however MTA-1 was cloned from the HTLV-I genome whereas K55 was cloned from HTLV-II. When the two recombinant proteins are immobilized to nitrocellulose for Western blotting, the K-55protein reacts with HTLV-II sera but not HTLV-I sera or sera from non-infected individuals as visible in ***Figure 10*** alongside MTA-1 reactivity. HTLV infections were verified by PCR analysis of blood cells.

Additionally, K-55 has demonstrated no cross immunoreactivity to sera from individuals infected with HIV, HBV, or HCV. All stated immunoreactivity has been confirmed through extensive in-house testing and qualification performed at MP Biomedicals Asia Pacific Pte Ltd during antigen verification and validation activities. Subsequent production analyses support the activity of the K-55 protein as immunoreactive to HTLV-II and non-immunoreactive to HTLV-I, and with demonstrated specificity in the presence of potentially cross-reactive *in vitro* substances.

4) HTLV-I Viral Lysate

HTLV-I Viral Lysate is a purified and inactivated native HTLV-I virus preparation derived from the HuT102 cell line. The intended use is as a raw material for manufacture of Western Blot analyses of HTLV infected sera.

The HuT102 cell line was derived from human T cells infected with HTLV-I. A PCR assay is used to verify HTLV-I presence as well as the absence of HIV-1, HIV-2 and HTLV-II. Cells cultured in standard medium shed the retrovirus. Viral disruption is carried out in Triton X-100 with 0.6M KCl buffer. The Triton is removed by double ether extraction. Viral inactivation is assessed by a reverse transcription (RT) assay. The protein concentration is assessed by the bicinchoninic acid (BCA) protein assay and quality control of lot batches is determined by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western blot comparison to reference lysates.

The HTLV-I Viral Lysate consists of several proteins produced from the *gag* region of the HTLV-I virus. These proteins are indicated in **Table 09.**

Table 09: HTLV-I Viral Lysate Gene Products

Viral Lysate Band	Gene Product
p53	Precursor of *gag* protein
gp46	*Envelope protein*
p36	*gag* protein intermediate
p32	*gag* protein intermediate
p28	*gag* protein intermediate
p26	*gag* protein intermediate
p24	**Major *gag* capsid protein**
p19	**Major *gag* matrix protein**

The HTLV Blot 2.4 kit primarily uses immunoreactivity to *gag* proteins p19 and p24 in HTLV infection confirmation and differentiation. Both p19 and p24 are reactive to antibodies from HTLV-I and cross-reactive to antibodies from HTLV-II, and can therefore be used to confirm infection. Furthermore, the relative intensity of each of these *gag* proteins can be used to differentiate between HTLV type 1 and type 2; the intensity of the p19 band is shown to be greater than or equal to that of the p24 band in HTLV type 1 specimens, and conversely, the intensity of the p24 band is shown to be greater than that of the p19 band in HTLV type 2 specimens[55] **(Reference Section 9d: Type Discrimination by p19, p24 Relative Intensity).**

f) Previous Clinical Studies

As described earlier, the HTLV Blot 2.4 is an improved version of the HTLV Blot 2.3, developed in 1995 and CE marked in 2004. The only change made from the HTLV Blot 2.3 to the HTLV Blot 2.4 was the substitution of the p21e protein with the more specific GD21.

The performance of the HTLV Blot 2.4 was evaluated in four clinical studies; three evaluations were conducted in France and one in Belgium. Some of the studies used IVD products HTLV Blot 2.3 and HTLV I/II ELISA 3.0 for the purpose of data comparison.

1) Evaluation of HTLV BLOT 2.4 performed by Universitaire Ziekenhuizen Leuven, Belgium

Fifteen (15) samples from Suriname, Africa, selected by positive results using the SERODIA HTLV (Fujirebio) particle agglutination assay, were tested with the HTLV Blot 2.4. Four of the fifteen were determined to be HTLV-I seropositive, six were negative and five were indeterminate. Of the five indeterminate samples, none had detectable reactivity with the GD21 antigen. In this small sample population, the SERODIA HTLV assay incorrectly identified 11/15 as HTLV seropositive. The HTLV Blot 2.4 determined the 4 HTLV-I positives with full profiles and strong intensity. The SERODIA HTLV assay was unable to differentiate HTLV-I and HTLV-II infections.

Thirty-four (34) samples from Belgian blood donors, identified as HTLV-I/II ELISA 3.0 repeat reactive and HTLV BLOT 2.3 indeterminate, were retested on the HTLV Blot 2.4. Recombinant gp21 (rgp21 or p21e) was falsely reactive with 26 of 34 samples on the HTLV Blot 2.3 whereas only one of the 34 remained weakly reactive with GD21 on the HTLV Blot 2.4.

2) Evaluation of HTLV Blot 2.3 vs. HTLV Blot 2.4 performed by Societie Francaise Centre Regional de Transfusion Sanguine (CRTS), France

Sera were classified into various groups as HTLV positive, HTLV negative, p19 isolates, p24 isolates, rgp21 isolates, rgp21 + p19 reactivities, rgp21 + p24 reactivities, and unclassified cases. Results of the comparison are shown in **Table 10** below.

Table 10: GD21 Specificity / Sensitivity

Pos/Neg/Ind	No. of samples	HTLV Blot 2.3	HTLV Blot 2.4
HTLV Positives	12	HTLV-I Pos = 12/12 **(100%)**	HTLV-I Pos = 12/12 **(100%)**
HTLV Negatives	5	HTLV Neg = 5/5 **(100%)**	HTLV Neg = 5/5 **(100%)**
Indeterminates	12	12/12 = **100%**	7/12 = **58%**
p19 only	2	Ind = 2/2	Ind = 2/2
p24 only	1	Ind = 1/1	Ind = 1/1
rgp21 only	6	Ind = 6/6	Ind = 1/6
rgp21 + p19	2	Ind = 2/2	Ind = 2/2
rgp21 + p24	1	Ind = 1/1	Ind = 1/1

The evaluation demonstrated that the HTLV Blot 2.4 sensitivity is equivalent to that of the HTLV Blot 2.3, detecting 12 out of 12 HTLV-I seropositives. The frequency of indeterminate results is significantly improved in this select population dropping from 100% to 58% due to the change to the GD21 protein.

3) In-house evaluation of HTLV BLOT 2.4 on the Montpellier Panel (#100 series)

The Montpellier Panel was obtained from the French National Blood Centre and consisted of a total of 45 members, comprising HTLV-I sera, HTLV-II sera, HTLV-I and HTLV-II dilution sera, and some p19 and p24 false positive samples. In this evaluation, the HTLV Blot 2.4 was run in parallel with the HTLV Blot 2.3 using the Montpellier panel, and the protocol specified in the respective Instruction for Use (IFU). The sensitivity of HTLV Blot 2.4 was comparable to that of HTLV Blot 2.3.

4) In-house evaluation using samples from Mdm Courouce (Societie Francaise de Transfusion Sanguine, SFTS), France

In order to determine the whether there was an improvement in specificity of HTLV Blot 2.4 over the HTLV Blot 2.3, sera, which were showing false reactivity to rgp21 (or p21e), were obtained from SFTS to run the assay in parallel. SFTS Panel comprised 28 members with five seropositive sera numbered 1 to 5 while the remainder were classified as indeterminate with reactivity to rgp21(or p21e). Results from the study demonstrated that, apart from the true HTLV seropositive samples (1-5), those sera which were showing false reactivity to rgp21 (or p21e) in the HTLV Blot 2.3 strips, did not give the same non-specific reaction in the HTLV Blot 2.4.

9. HTLV BLOT 2.4 INTERPRETATION CRITERIA

a) Overview of HTLV Blot 2.4 Criteria

The HTLV Blot 2.4 interpretation criteria is based on the presence or absence of five key protein markers, recombinant proteins GD21, rgp46-I (MTA-1), and rgp46-II (K-55), and native viral proteins p19 and p24. The immunoreactivity of each of these proteins is indicated in **Table 11** below. All five protein markers are employed in the HTLV Blot 2.4 to confirm and differentiate HTLV infection.

Table 11: Immunoreactivity of HTLV Blot 2.4 Key Antigens

Antigen	Immunoreactivity
GD21	Immunoreactivity to HTLV-I and HTLV-II
rgp46-I (MTA-1)	Immunoreactivity to HTLV-I
rgp46-II (K-550	Immunoreactivity to HTLV-II
p19	Immunoreactivity to HTLV-I and HTLV-II
p24	Immunoreactivity to HTLV-I and HTLV-II

In addition to the known confirmatory immunoreactivity of p19 and p24, the differential reactivity as measured by their relative intensity is used to differentiate between HTLV type 1 and type 2 infection in the absence of any reactivity to the type specific recombinant antigens (i.e. rgp46-I and rgp46-II). In HTLV type 1 samples, the relative intensity of p19 is greater than or equal to that of p24; conversely, in HTLV type 2 samples, the relative intensity of p24 is greater than that of p19[54].

The native HTLV-I viral lysate contains proteins in addition to the aforementioned p19 and p24. The majority of these proteins is classified as *gag* protein intermediates or precursors and includes the following: p26, p28, p32, p36, p53. Additionally, the viral lysate contains gp46, an *env* protein, which is often diffuse in appearance when present, and in HTLV-I samples only. As discussed in detail in **Section 9c: HTLV Gag Indeterminate Profile/Gag Indeterminate Profiles,** these proteins are not used in confirmation or discrimination, but are only taken into account in determining seronegative status. Research has demonstrated that specific combinations of these *gag* proteins may be present in sera from individuals shown to be uninfected with HTLV type 1 or type 2 by PCR, clinical diagnosis, and / or donor follow-up. Serological profiles of these specific *gag* proteins classified as seronegative by the criteria of the HTLV Blot 2.4 include the following:

- Presence of any combination of *gag* proteins without p24 (i.e. p26, p28, p32, p36, p53); and
- Any individual *gag* protein, including p24.

The HTLV Blot 2.4 combines the immunoreactivity of the HTLV antigens included in the kit into an interpretation criteria as indicated in **Table 12.** This interpretation criterion has been utilized as part of the HTLV Blot 2.4 assay since the CE mark was obtained in 2004.

Table 12: HTLV Blot 2.4 Interpretation Criteria

Seronegative	• No reactivity to HTLV specific proteins; or • Any combination of *gag* proteins excluding p24 (i.e. p19, p26, p28, p32, p36, p53); or • Any single *gag* protein, inclusive of p24
HTLV-I Seropositive	• Reactivity to p19, GD21 ***and*** rgp46-I: or • Reactivity to p19, p24 ***and*** GD21, with reactivity to p19 greater than or equal to p24
HTLV-II Seropositive	• Reactivity to p24, GD21 ***and*** rgp46-II: or • Reactivity to p19, p24 ***and*** GD21, with reactivity to p24 greater than p19
HTLV-I/II Seropositive	• Reactivity to GD21, p19, p24, rgp46-II ***and*** rgp46-I
Indeterminate	• Reactivity to HTLV specific bands that do not meet the criteria for HTLV-I seropositive, HTLV-II seropositive, HTLV-I/II seropositive or seronegative

b) Differences between HTLV Blot 2.4 Criteria and Current Guidelines

The HTLV Blot 2.4 interpretation criteria, as described above, has been in use since the product was first CE marked in 2004. This interpretation criteria differs from the guidelines used by different organizations such as the Center for Disease Control and Prevention (CDC), World Health Organization (WHO), and U.S. Public Health Service (USPHS) working group. MP Biomedicals considers these guidelines to be outdated as significant advances have been made since the testing of HTLV began in 1988 that are not incorporated. In the last version of HTLV counseling guidelines from the CDC[56], the definition of a confirmed positive HTLV specimen was based on the criteria for HTLV-I/II seropositivity adopted by the USPHS working group in 1988. This criteria states: *"a specimen that is repeatedly reactive by enzyme immunoassay must demonstrate immunoreactivity to both the gag gene product p24 and to an env gene product (gp46 and /or gp61/68) to be considered seropositive for HTLV-I/II. Reactive serum specimens that do not satisfy these criteria but do show immunoreactivity to at least one suspected HTLV gene product are designated "indeterminate." Serum specimens with no immunoreactivity to any HTLV gene product in additional, more specific tests are considered false positive."* This original interpretative criteria for HTLV-I and HTLV-II supplemental tests was developed in 1988 when the only available supplemental products were developed from native viral proteins derived from viral lysate. In fact, the first iteration of the HTLV Blot, the HTLV Blot 1.2, was developed around the same time and utilized native viral proteins alone.

The 1988 CDC interpretative criteria did not recognize the development of the more type specific recombinant *env* proteins. What it did recognize was the first version of a recombinant *env* protein for HTLV detection, p21e, now associated with a high level of false reactivity. *"An important advance in HTLV serologic testing has been the development of a recombinant env protein, p21e. Reactivity to p21e (in either enzyme immunoassay or "spiked" Western immunoblot) has been found to be highly sensitive for HTLV-I/II infection, being observed in virtually 100% of infected persons. However, the specificity of the p21e reactivity has been questioned. For purposes of notification and counseling, the positivity of samples showing p21e serologically should be confirmed by tests that detect env reactivity, such as radioimmunoprecipitation or recombinant protein-based assays, or by polymerase chain reaction until further information is available concerning this test."* As noted earlier, the second iteration of the HTLV Blot, the HTLV-I Blot 2.0, incorporated the use of p21e.

Today, the HTLV Blot 2.4 uses a further refinement of p21e, or GD21, to reduce the false positivity associated with the use of p21e. Additionally, recombinant antigens specific to HTLV-I and HTLV-II, MTA-1 and K-55 respectively, are now available and used as part of the assay. With advances in technology since 1988-1993, it appears that the HTLV interpretative criteria require revision recognizing that viral lysate will only be a small component of a product, and used in concentrations sufficient only for the detection of viral *gag* antibodies, not native viral *env* antibodies. In addition, the use of HTLV recombinant antigens has progressed significantly, resulting in high sensitivity, specificity, and viral differentiation.

The HTLV interpretative guidelines as defined by the major organizations throughout the years are indicated in **Table 13.**

Table 13: HTLV Interpretative Guidelines by Organization

Organization	Year	Positive	Negative	Indeterminate
USPHS, WHO, CDC[56]	1988	**HTLV:** p24 and (gp46 or gp61/68)	No HTLV bands	Any other band profiles
CRSS	1988	**HTLV:** p19 or p24 and (gp46 or gp61/68)	No HTLV bands	Any other band profiles
WHO[57]	1990	**HTLV:** One *gag* (p19, p24) and one *env* band (gp46, gp61/68)	No HTLV bands	Any other band profiles
ASTPHLD[59]	1991	**HTLV:** p19 or p24 and one env band (gp21, gp46, gp61/68)	No HTLV band	Other band profiles
WHO, CDC[58]	1993	**HTLV-I:** p19 or p24 and any of 3 env bands (gp21e, gp 46, gp61/68) **HTLV-II:** no criteria established yet	No HTLV band	Other band profiles
HTLV European Research Network	1996	**HTLV-I:** p19, p24, rgp21 and gp46-I **HTLV-II:** p24, rpg21, rpg46-II	No HTLV band	Other band profiles

ASTPHLD = Association of State and Territorial Public Health Laboratory Directors

c) HTLV GAG Indeterminate Profile / GAG Indeterminate Profiles

HTLV screening assays are sensitive but not specific in their detection of HTLV-I/II antibodies, resulting in the development of HTLV-I/II seropositivity criteria for supplemental HTLV assays designed to confirm and discriminate the presence of antibodies to HTLV. As indicated in **Table 13,** existing guidelines for seronegativity involve a complete lack of HTLV bands; this stringent criteria results in a high indeterminate rate for Western blot assays, as donor specimens may show reactivity to isolated *gag* proteins. This reactivity to isolated *gag* proteins was demonstrated in analyses of many sera collected from tropical regions, which exhibited different HTLV patterns[60,61], many of which showed reactivity to these isolated *gag* proteins[62,63]. Among the different profiles seen in reactivity to *gag* proteins is a profile designated as HTLV-I Gag Indeterminate Profile (HGIP)[68], and is the most frequent profile seen in Central Africa. HGIP exhibits intense HTLV-I seroreactivity with patterned immunoreactivity to *gag* proteins p19, p26, p28, with or without p32, with or without p36, and p53, but no immunoreactivity to p24 or any *env* glycoproteins such as gp21, gp46 or MTA-1[61].

The reactivity of sera to *gag* proteins varies according to HTLV-I/II endemicity (i.e. to the geographical area studied). Among blood donors in areas of low endemicity (i.e. Europe and the U.S.), the *gag* profiles consist of faint isolated *gag* immunoreactivity[69,70]. They occur at a frequency similar to true HTLV-I seropositivity ranging from 0 to 0.022% among blood donors. In such populations, HGIP appears to be very rare[71]. Although some uncertainty remains, donors with these indeterminate *gag* immunoreactivity profiles are generally counseled that they are not infected with HTLV[72,73,74,75,76,77]. By contrast, in tropical areas such as Central Africa, Melanesia, and some regions of Southeast Asia and South America, the prevalence rates of the *gag* immunoreactivities is high, representing in some cases more than 50% of all Western blot profiles[78,79]. In these *gag* profiles, HGIP makes up a large proportion, approximately 41 to 42%[79,80]. It was suggested that the frequent HGIP seen in the Western blot results was not caused by HTLV-I infection but instead could be associated with cross reactivity to other proteins. Follow-up on individuals infected with HGIP demonstrated that the Western blot profiles did not evolve over time and no case of HTLV-I seroconversion could be documented by studying sequential samples[65]. HTLV-I proviral sequences were not detected by PCR in the peripheral blood mononuclear cell (PBMC) DNA, thereby strongly suggesting that an HGIP does not reflect true HTLV-I infection. In this regard, healthy blood donors with HGIP should be reassured that they are unlikely to be infeted with HTLV-I or HTLV-II.

For the vast majority of indeterminate samples originating from tropical areas, it is hypothesized that the indeterminate reactivity was the result of sequence homologies between *gag* epitopes and HTLV-I and other proteins. For example, antibodies to the blood stage antigens of Plasmodium *falciparum* (malaria) were suggested to cross-react with an HTLV p19 epitope, leading to the presence of *gag* immunoreactivities in specimens from areas where malaria is endemic, such as the Philipines, Papua New Guinea, Indonesia, and Brazil[64,65,66,67].

A comprehensive six year study by Rouet et al[78] prospectively assessed 37,724 Caribbean blood donors for the presence of immunoreactivity to specific HTLV antigens. It was concluded that, on the basis of data gathered, and by analogy with HTLV-II seropositivity criteria that requires reactivity to only three bands (i.e. GD21, p24 and rgp46-II), that HTLV-I seropositivity should be similarly based on the presence of at least immunoreactivity to three bands (i.e. GD21, p19 and rgp46-I), even if p24 is lacking. The study found that when both rgp46-I and p24, or the *env* protein immunoreactivities are lacking, as in HGIP, HTLV-I proviral DNA is not detected by PCR in all cases.

The MP Diagnostics HTLV Blot 2.4 incorporates the findings above into its interpretation criteria, considering reactivities to *gag* profiles, including HGIP, combination *gag* profiles without p24, and single *gag* profiles including p24, as seronegative.

d) Type Discrimination by p19, p24 Relative Intensity

In the absence of a type specific recombinant protein (i.e. rgp46-II or rgp46-I), HTLV type discrimination can be made based on the relative intensities of p19 and p24. In HTLV-I type 1 specimens, the relative intensity of p19 is greater than or equal to that of HTLV-II; conversely, in HTLV type 2 specimens, the relative intensity of p24 is greater than that of p19[55.]

A study by Lal[81] et al looked at the relative intensities of the p19 and p24 *gag* proteins as a method of serological discrimination. PCR confirmed serum specimens were analyzed using Western blot methodology and the viral type discriminated on the basis of p19 and p24 relative intensities alone, with HTLV-I defined as p19 ≥ p24, and HTLV-II as p24 > p19. Of the 60 PCR confirmed HTLV-I samples, 56 (93%) had a relative intensity of p19 that was greater than that of p24. Of the 61 PCR confirmed HTLV-II samples, 56 (92%) had a relative intensity of p24 that was greater than that of p19.

The MP Diagnostics HTLV Blot 2.4 incorporates the findings above into its interpretation criteria, allowing for HTLV type differentiation on the basis of the relative intensities of p19 and p24 in the absence of reactivity to an HTLV type specific recombinant.

e) Overview of U.S. Clinical Study

The performance of the HTLV Blot 2.4 overall, as well as its interpretation criteria, was validated as part of the U.S. clinical studies. Clinical study MP-EIA-HTLV-001B was a retrospective study designed to assess the validity and reproducibility of the MP Diagnostics HTLV Blot 2.4 Western Blot Assay, automated using the MP Diagnostics AutoBlot System 20, as compared to that of a non-reference standard, the CDPHL algorithm. The validity of the HTLV Blot 2.4 assay was assessed using two population types: 1) specimens that had screened HTLV non-reactive using a licensed screening assay; and 2) specimens that screened repeatedly reactive (RR) on two screening assays but were unconfirmed positives. Additionally, a sensitivity estimation study was planned that would assess the performance of

the HTLV 2.4 in an HTLV known positive population. The study was conducted at three geographically distinct sites: the American Red Cross (ARC) National Testing Laboratory (NTL) confirmatory laboratory in Charlotte, NC; the California Department of Public Health Laboratories in Richmond, CA; and LABS, Inc., in St. Louis, MO. The reproducibility of the HTLV Blot 2.4 was evaluated by testing aliquots of a 3-member HTLV panel (HTLV-I, HTLV-II, and negative) over multiple lots, clinical testing sites, operators, and replicates. The results of the clinical study demonstrated the validity of the HTLV Blot 2.4 interpretation criteria; all results are discussed in detail in **Section 10: Clinical Study Results.**

10. CLINICAL STUDY RESULTS

a) Reproducibility

The reproducibility of the HTLV Blot 2.4 was evaluated using a three-member panel tested in duplicate over multiple lots, clinical testing sites, and with different operators. The three-member consisted of the following specimens: HTLV- antibody specimen; HTLV-II antibody specimen; and one specimen non reactive for anti-HTLV-I/HTLV-II. The HTLV-I and THLV-II panel members were obtained by pooling archived HTLV-I and HTLV-II donor specimens respectively until sufficient volume was obtained. Each panel member was assayed in duplicate over three lots of HTLV Blot 2.4 product, by three different operators, at each of the clinical sites; a total of 54 test strips per panel member were analyzed as part of this study.

Reproducibility was assessed using percent agreement on positivity/negativity, defined as the number of comparisons between two runs that agree, regardless of whether or not they agree with actual specimen status, divided by the total number of pairwise comparisons made. Assessments were evaluated for each panel member separately, and percent agreement calculated for between replicate (within operator), within site, within lot, and overall. 95% confidence intervals (CIs) were calculated for each level of percentage agreement.

In this study, no strips were incorrectly interpreted. In addition, the within operator agreement was calculated as 100%; the within site agreement as 100% with a one-sided lower limit of 63.1%; the within lot agreement as 100% with a one-sided lower limit of 95%; and the between lot agreement as 100% with a one-sided lower limit of 99.1%. These data demonstrate that the MP Diagnostics HTLV Blot 2.4 assay is reproducible across multiple replicates, operators, sites, and lots.

b) Known Positive Samples

The sensitivity of the HTLV Blot 2.4 was evaluated using a Known Positive Population. Two-hundred (200) specimens were obtained from the well-characterized ARC repository, and were aliquots of plasma units from donors that had previously screened repeatedly reactive using the licensed Abbott PRISM HTLV-I/HTLV-II ChLIA assay and were confirmed as infected with HTLV-I or HTLV-II by the unlicensed CDPHL HTLV Supplemental Testing Algorithm. The 200 Known Positive samples were divided between the ARC Charlotte NTL, LABS, Inc., and CDPHL for evaluation on the HTLV Blot 2.4; testing was performed using three product lots. All specimens were tested using the HTLV Blot 2.4 assay using the defined HTLV Blot 2.4 interpretation criteria, and the CDPHL Algorithm, which was performed until a definitive answer was obtained, and all individual HTLV Blot 2.4 results were compared to that of the CDPHL Algorithm. The reference standard was considered the test of record (TOR), which was the known positive result, and sensitivity was calculated as the number of specimens that test positive to the number of true positive specimens as per the reference standard. The status of HTLV infection was considered confirmed for all samples in this population, and the objective of testing a known positive population was to ensure that the HTLV Blot 2.4 did not miss any positive samples, thereby increasing the risk of contaminated blood samples, impacting donor notification and/or treatment, or increasing the risk of additional disease transmission.

Of the 200 samples tested, the HTLV Blot 2.4 identified 195 as positive based on the existing interpretation criteria as defined; 4 samples were identified as indeterminate, and one as negative, based on the same criteria. Of the 195 positive samples, 82 (42.1%) were identified as HTLV-I seropositive, 103 (52.8%) as HTLV-II seropositive, and 10 (5.1%) as HTLV-I/II seropositive. The CDPHL Algorithm identified 193 samples as positive, one as indeterminate, and six as negative. The sensitivity of each assay with the TOR is reported with an exact 95% CI**.** **Table 14**, calculated with indeterminate results as negative, reports the sensitivity of the HTLV Blot 2.4 with the TOR as 97.50%, with a 95% CI of 94.26% to 99.18%. Comparatively, the sensitivity of the CDPHL Algorithm with the TOR when indeterminates are considered negative is 96.50% (95% CI = 92.92%), 98.58%) and 97.00% (95% CI = 93.58%, 98.89%) when indeterminates are considered positive. The sensitivity of the HTLV Blot 2.4 was expected to be a minimum of 90%, which would have yielded a 95% CI lower bound of 85% for the sample size tested; the observed sensitivity of 97.50% exceeds the minimum expected result as defined.

Table 14: Known Positive Population Sensitivity

		CDPHL Algorithm			
		Positive	***Indeterminate***	***Negative***	***Total***
MP HTLV Blot 2.4	***Positive***	190	1	4	195 (97.50%)
	Indeterminate	3	0	1	4 (2.0%)
	Negative	0	0	1	1 (0.5%)
	Total	193 (96.50%)	1(0.5%)	6 (3.0%)	200

Common HTLV viral band patterns for the HTLV-I and HTLV-II samples are identified in **Table 15** and **Table 16** respectively.

Table 15: Known Positive Population HTL V-I Band Patterns (n=82)

HTLV Blot 2.4 Band Pattern	Frequency
GD21, **p19**, p24, p26, p28, p32, p36, gp46, p53, **rgp46-I**	44 (53.7%)
GD21, **p19**, p24, p28, p32, gp46, p53, **rgp46-I**	24 (29.3%)
GD21, **p19**, p24, p26, p28, p36, **rgp46-I**	4 (4.8%)
GD21, **p19**, p24, p36, **rgp46-I**	2 (2.4%)
GD21, **p19**, **rgp46-I**	2 (2.4%)
GD21, **p19**, **p24**	1 (1.2%)
GD21, **p19**, **p24**, p26, p28, p32, p36	1 (1.2%)
GD21, **p19**, **p24**, p26, p28, p32, gp46, p53	1 (1.2%)
GD21, **p19**, p24, p26, p28, p32, **rgp46-I**	1 (1.2%)
GD21, **p19**, p24, p28, p32, p36, **rgp46-I**	1 (1.2%)
GD21, **p19**, p24, p28, p32, p36, gp46, p53, **rgp46-I**	1 (1.2%)

Table 16: Known Positive Population HTLV-II Band Patterns (n=103)

HTLV Blot 2.4 Band Pattern	Frequency
GD21, p24, rgp46-II	31 (30.0%)
GD21, p19, p24, rgp46-II	28 (27.2%)
GD21, p19, p24, p53, rgp46-II	15 (14.6%)
GD21, p19, p24, p36, p53, rgp46-II	10 (9.7%)
GD21, p19, p24, p36, rgp46-II	10 (9.7%)
GD21, p24, p36, rgp46-II	3 (2.9%)
GD21, p24, p36, p53, rgp46-II	2 (1.9%)
GD21, p19, p24	1 (1.0%)
GD21, p19, p24, p26, rgp46-II	1 (1.0%)
GD21, p19, p24, gp46, p53, rgp46-II	1 (1.0%)
GD21, p19, p24, p28, p32, p36, rgp46-II	1 (1.0%)

The HTLV Blot 2.4 and the CDPHL both identified one sample from the known positive population as negative. Sample 1703 showed no HTLV specific bands when tested with the HTLV Blot and, while reactive on the CDPHL ELISA, was non-reactive on the IFA and Western blot testing performed as part of the CPDHL Algorithm.

As stated earlier, the testing results used to enroll samples in this study were from undiluted samples collected from the donor at the time of collection. The actual samples enrolled in the study were aliquots of plasma units diluted approximately 14% with anticoagulant; dilution of the aliquots would minimize the validity of a one-to-one comparison but the dilution effects of the samples on the testing results were considered insignificant at the time of enrollment. The discordance in the known positive state and the actual test result may have been due to dilution of the HTLV analyte in the trial sample to an undetectable range, or due to interference as a result of high levels of anticoagulant. It is proposed that the negative sample result is primarily due to the dilution of the analyte, as all trial samples had the same level of anticoagulants, and interferent effects were not visible in any other samples. Additionally, all original qualification samples had been confirmed as positive using the CDPHL Algorithm; the fact that the CDPHL Algorithm was unable to confirm the diluted aliquots supports the cause of the discordance as dilution.

1) HTLV Blot 2.4 / CDPHL Discordant Samples (Seropositivity)

Of the 200 known positive samples tested, HTLV Blot 2.4 and CDPHL results were discordant for nine samples in terms of seropositivity versus seronegativity. This analysis specifically looked at the ability of an assay to call a positive a positive, but not necessarily differentiate between viral types. A breakdown of the testing results for these nine samples is included in **Table 17.**

Table 17: Known Positive Population HTLV Blot 2.4 Discordant Samples (Seropositivity)

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1845	GD21, p19, p24, rgp46-II	HTLV -II	0.96				NEG
1864	GD21, p24, rgp46-II	HTLV -II	0.83				NEG
1676	GD21, p24	IND	2.31/2.06/2.25	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1682	GD21, p24	IND	1.90/2.38/2.30	HTLV-I (1:16) HTLV-II (1:64)			HTLV-II
1688	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	12.07/12.88/13.33	Inconclusive	p19, p28, p36, p21e, p53	Non-reactive	IND
1701	GD21	IND	3.74/3.83/4.03	Non-Reactive	p19		NEG
1744	GD21, rgp46-I	IND	1.77/1.40/1.46	HTLV-I (1:16) HTLV-II (1:8)			HTLV-I
1792	GD21, p24, rgp46-II	HTLV-II	0.56				NEG
1800	GD21, p19, p24, rgp46-II	HTLV-II	1.60/1.80/1.86	Non-reactive	P24		NEG

Five of nine of these discordant samples were discordant due to a negative final interpretation on the part of the CDPHL Algorithm (samples 1845, 1864, 1701, 1792, and 1800). With the exception of sample 1701, which was resulted as indeterminate with the HTLV Blot 2.4, the HTLV Blot identified all the CDPHL negative samples as HTLV-I or HTLV-II seropositive. Three of the five CDPHL negatives were below the detection range of the ELISA and did not proceed to additional, supplemental testing. The other two samples were ELISA reactive, but IFA and Western blot non-reactive. An additional sixth

discordant sample, sample 1688, was HTLV Blot 2.4 HTLV-I seropositive and CDPHL indeterminate, despite testing on multiple assays, the IFA, the Western blot and the RIPA. As seen in **Table 17,** the HTLV Blot 2.4 assay produced a full HTLV-I seropositive profile. The HTLV Blot 2.4 therefore shows increased sensitivity in detecting low level samples as compared to the CDPHL HTLV Algorithm.

Out of the 200 samples tested as part of the known positive population, the HTLV Blot 2.4 identified only 4 as indeterminate, for an overall indeterminate rate of 2.0%. The indeterminates in the known positive population were analyzed for indeterminate patterns**;** the results are depicted in **Table 18** below.

Table 18: Known Positive Population Indeterminate Band Patterns (n=4)

HTLV Blot 2.4 Band Pattern	Frequency
GD21	1 (25.0%)
GD21,p24	2 (50.0%)
GD21, rgp46-I	1 (25.0%)

2) HTLV Blot 2.4 / CDPHL Discordant Samples (HTLV Viral Type Discrimination)

The HTLV Blot 2.4 interpretation criteria utilizes the presence of HTLV specific bands in various combinations and intensities to confirm and differentiate between HTLV-I seropositive, HTLV-II seropositive and HTLV-I/II seropositive. In addition to the overall accuracy of the HTLV Blot 2.4 in identifying a positive sample, the accuracy of the interpretation criteria in differentiating viral types was assessed. For the majority of the positive samples, the HTLV Blot 2.4 was concordant with the viral type discrimination as presented by the CDPHL Algorithm; only 11 of the concordant positive samples differed in viral type discrimination. **Table 19** lists the 11 samples that differed according to viral type discrimination.

Table 19: Known Positive Population HTLV Blot 2.4 Discordant Samples (HTLV Viral Type Discrimination)

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1832	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-II, rgp46-I	HTLV –I/II	15.48/14.60/ 14.57	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1841	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-II, rgp46-I	HTLV –I/II	9.62/9.30/9.4 8	HTLV-I (1:512) HTLV-II (1:256)			HTLV-I
1844	GD21, p19, p24, rgp46-II, rgp46-I	HTLV –I/II	1.80/1.60/1.6 8	HTLV-I (1:16) HTLV-II (1:64)			HTLV -II
1866	GD21, p19, p24,	HTLV -I	1.08/1.29/1.4 1	HTLV-I (1:8) HTLV-II (1:32)			HTLV -II
1683	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	9.78/9.06/9.2 7	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1695	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	21.73/23.70/ 23.76	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I

Table 19: Known Positive Population HTLV Blot 2.4 Discordant Samples (HTLV Viral Type Discrimination)

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1755	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	14.34/13.74/ 13.61	HTLV-I (1:4096) HTLV-II (1:2048)			HTLV-I
1758	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	12.05/11.70/ 11.93	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1775	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	25.04/27.19/ 26.06	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1776	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	13.25/11.45/ 11.03	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1779	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	14.45/14.78/ 15.28	HTLV-I (1:512) HTLV-II (1:256)			HTLV-I

Ten out of the eleven samples were discordant due to a result of HTLV-I/II seropositivity, which is a final result interpretation that the CPDHL Algorithm does not offer. A sample is HTLV-I/II seropositive when reactivity to p19, p24, GD21, rgp46-II and rgp46-I is observed. This band pattern can be present with or without the Non-Critical Gag Proteins (NCGP). Common HTLV-I/II seropositive band patterns identified in the known positive population are depicted in **Table 20.**

Table 20: Known Positive Population HTLV-I/II Band Patterns (n=10)

HTLV Blot 2.4 Band Pattern	Frequency
GD21, p19, p24, p26, p28, p36, gp46, p53, rgp46-II, rgp46-I	5 (50%)
GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	4 (40%)
GD21, p19, p24, rgp46-II, rgp46-I	1 (10%)

Of the 200 known positive samples tested, the HTLV Blot 2.4 and CDPHL Algorithm were concordant for 90% of samples in terms of HTLV viral type discrimination. This concordant rate attests to the validity of the HTLV Blot 2.4 interpretation criteria. The HTLV Blot 2.4 uses a combination of band patterns to discriminate between HTLV-I and HTLV-II type infections. The main criteria for discrimination between viral type infections is the presence of MTA-1 (rgp46-I) or K55 (rgp46-II) in an HTLV sample. MTA-1 is a recombinant antigen specific to portions of the HTLV-I virus, and K55 a recombinant antigen specific to HTLV-II. Presence or absence of a specific recombinant is the first step in discrimination. Additionally, the presence of *gag* proteins contained within the viral lysate, namely p19 and p24, can be used to discriminate between infection types. The HTLV Blot 2.4 outperformed the CDPHL Algorithm in identifying positive samples, especially low-level positive samples. This supports the use of the HTLV Blot 2.4 as a confirmatory assay and demonstrates the sensitivity of the HTLV Blot 2.4 in identifying positive samples

c) HTLV Screening Nonreactive Population

The comparison between the HTLV Blot 2.4 assay and the CDPHL Algorithm on percent negative agreement with the TOR was evaluated by testing 200 HTLV screening negative specimens, herein EIA negative population. These samples were qualified as testing previously non-reactive using a licensed HTLV screening assay; the licensed screening assay results were considered the non-reference standard and listed as the test of record (TOR) for this sample population. These specimens were obtained from the well-characterized ARC repository and were aliquots of plasma units that had previously screened negative for all ARC blood donor-screening markers. The 200 EIA Negative samples were divided among the ARC Charlotte NTL, LABS, Inc., and CDPHL for evaluation on the HTLV Blot 2.4; testing was performed using three product lots. All specimens were tested by the HTLV Blot 2.4 assay and the CDPHL Algorithm, and all individual HTLV Blot 2.4 results were compared to that of the CDPHL Algorithm. Percent negative agreement of the HTLV Blot 2.4 and the CDPHL HTLV Algorithm with the TOR was calculated.

No samples were identified as positive (or reactive) by either the HTLV Blot 2.4 or the CDPHL algorithm; therefore, there were no false positives by either assay as defined by population type.

Of the 200 samples tested, the HTLV Blot 2.4 identified 169 as negative based on the interpretation criteria; 31 samples were identified as indeterminate based on the same criteria. The CDPHL Algorithm identified all 200 samples as negative. Percent agreement of each assay with the TOR is reported with an exact 95% confidence interval (CI)**.** The percent negative agreement of the HTLV Blot 2.4, calculated with indeterminate results as negative, reports the percentage negative agreement of the HTLV Blot 2.4 with the TOR as 100%, with a 95% CI of 98.17% to 100%. Comparatively, the percent negative agreement of the CDPHL Algorithm with the TOR is 100% (95% CI = 98.17% to 100%). **Table 21** depicts the agreement of the HTLV Blot 2.4 test results with that of the CDPHL Algorithm.

Table 21: EIA Negative Population Results

		CDPHL Algorithm			
		Positive	***Indeterminate***	***Negative***	***Total***
MP HTLV Blot 2.4	***Positive***	0	0	0	0
	Indeterminate	0	0	31	31 (15.5%)
	Negative	0	0	169	169 (84.5%)
	Total	0	0	200	200 (100%)

The major band patterns of the negative samples as identified by the HTLV Blot 2.4 are indicated in **Table 22** below.

Table 22: EIA Negative Population HTLV Blot 2.4 Negative Band Patterns (n = 169)

HTLV Blot 2.4 Band Pattern	Frequency
Clean (no bands)	157 (92.9%)
p24	8 (4.7%)
p19	4 (2.4%)

Of the 200 samples tested by the CDPHL Algorithm, 16 of these were initially repeatedly reactive (RR) on the CDPHL HTLV in-house ELISA and were subjected to supplemental testing as part of the CDPHL Algorithm. All 16 were non-reactive on the CPDHL IFA, but still required additional testing to complete the algorithm; three samples went all the way through the algorithm to RIPA before they were reported out as negative. One of these samples, sample 2079, was CDPHL western blot reactive with a p21e band, but completely negative by HTLV Blot 2.4, attesting to the specificity of the GD21 band over its p21e counterpart.

Individual subsections of these data are discussed below. Overall, the results from the negative population support the ability of the HTLV Blot to identify and confirm HTLV negative donor samples.

1) HTLV Blot 2.4 Indeterminate Results / CDPHL Algorithm Discordant Results

Of the 200 samples tested as part of the EIA negative population, the HTLV Blot 2.4 called 31 of these as indeterminate. These indeterminate samples were the source of discordance between the HTLV Blot 2.4 and the CDPHL Algorithm when indeterminate results were considered positive, contributed to the percent negative agreement calculation of 84.50% and will therefore be discussed before concordant results. A detailed results summary for all HTLV Blot 2.4 indeterminate samples is indicated in **Table 23** below.

Table 23: EIA Negative Population HTLV Blot 2.4 Indeterminate Sample Results

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2017	GD21	IND	0.33				NEG
2021	GD21	IND	0.33				NEG
2024	GD21	IND	0.34				NEG
2029	GD21	IND	0.66				NEG
2030	GD21	IND	0.27				NEG
2032	rgp46-I, rgp46-II	IND	0.83				NEG
2039	GD21	IND	0.26				NEG
2042	GD21	IND	0.44				NEG
2043	GD21	IND	0.44				NEG
2052	GD21	IND	0.22				NEG
2055	GD21	IND	1.10/0.89/1.00	Nonreactive	Nonreactive		NEG
2056	GD21, rgp46-II	IND	0.50				NEG
2060	GD21	IND	0.26				NEG
2062	GD21, p24	IND	0.39				NEG
2063	GD21	IND	0.49				NEG
2065	GD21	IND	0.37				NEG
2072	GD21	IND	0.35				NEG
2074	GD21	IND	0.43				NEG
1902	GD21	IND	1.21/1.10/1.16	Non-Reactive	Non-Reactive		NEG
1903	GD21	IND	0.53				NEG
1904	rgp46-II	IND	0.38				NEG
1905	GD21, p24	IND	0.27				NEG
1913	GD21	IND	0.32				NEG
1943	GD21	IND	0.01				NEG
1945	GD21	IND	0.32				NEG
1951	rgp46-I, rgp46-II	IND	0.21				NEG
1953	rgp46-II	IND	0.27				NEG
1954	rgp46-II	IND	0.26				NEG
1959	GD21, p24	IND	0.32				NEG
1960	rgp46-II	IND	0.30				NEG
2001	rgp46-I	IND	0.17				NEG

All of the samples resulted as indeterminate by the HTLV Blot 2.4 showed reactivity to one of more of the HTLV specific recombinant antigens. The frequency of recombinant antigen binding, along with the other viral protein markers, is indicated in **Table 24.** The highest banding frequency in an indeterminate sample was shown with GD21 alone, twenty samples for a frequency of 64.5%.

Table 24: EIA Negative Population HTLV Blot 2.4 Indeterminate Band Patterns (n = 31)

HTLV Blot 2.4 Band Pattern	Frequency
GD21	20 (64.5%)
rgp46-II	4 (12.9%)
GD21, p24	3 (9.7%)
rgp46-II, rgp46-I	2 (6.5%)
GD21, rgp46-II	1 (3.2%)
rgp46-I	1 (3.2%)

2) HTLV Blot 2.4 / CDPHL Concordant Negative Results

Of the 200 samples in the EIA negative population, the HTLV Blot 2.4 resulted out 169 as negative. These 169 sample results were concordant with the CDPHL Algorithm overall interpretation, for a total percent negative agreement of 84.50% (95% CI = 78.73%, 89.22%) with the TOR. The HTLV Blot 2.4 band patterns for the concordant negative population are tabulated along with the CDPHL Algorithm results in **Table 25.**

Table 25: EIA Negative Population HTLV Blot 2.4 / CDPHL Algorithm Concordant Results

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2014		NEG	0.57				NEG
2015		NEG	0.38				NEG
2016		NEG	0.41				NEG
2018		NEG	2.73/3.28/3.53	Nonreactive	Nonreactive		NEG
2019		NEG	0.27				NEG
2020		NEG	0.24				NEG
2022		NEG	0.27				NEG
2023		NEG	0.47				NEG
2025		NEG	0.59				NEG
2026	p19	NEG	0.49				NEG
2027		NEG	0.30				NEG
2028		NEG	0.35				NEG
2031		NEG	0.45				NEG
2033		NEG	0.33				NEG
2034		NEG	0.33				NEG
2035		NEG	0.76				NEG
2036		NEG	0.91				NEG
2037		NEG	0.80				NEG
2038		NEG	1.02/1.22/1.27	Nonreactive	Nonreactive		NEG
2040		NEG	0.57				NEG
2041		NEG	1.57/1.38/1.46	Nonreactive	Nonreactive		NEG
2044		NEG	0.37				NEG
2045		NEG	2.29/2.26/2.36	Nonreactive	Nonreactive		NEG
2046		NEG	0.45				NEG
2047		NEG	0.33				NEG
2048		NEG	0.18				NEG
2049		NEG	0.51				NEG
2050		NEG	0.43				NEG
2051		NEG	0.35				NEG
2053		NEG	0.30				NEG
2054		NEG	0.29				NEG
2057		NEG	0.43				NEG
2058		NEG	0.36				NEG
2059		NEG	0.37				NEG
2061		NEG	0.45				NEG
2064	p24	NEG	0.24				NEG
2066		NEG	0.46				NEG
2067		NEG	0.16				NEG
2068		NEG	0.34				NEG
2069		NEG	0.35				NEG
2070		NEG	0.36				NEG
2071		NEG	0.79				NEG
2073		NEG	0.54				NEG

Table 25: EIA Negative Population HTLV Blot 2.4 / CDPHL Algorithm Concordant Results

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2075		NEG	11.20/9.76/8.02	Nonreactive	Nonreactive		NEG
2076		NEG	0.44				NEG
2077		NEG	0.93				NEG
2078		NEG	0.32				NEG
2079		NEG	1.63/1.83/1.81	Nonreactive	P21e	Nonreactive	NEG
1878		NEG	0.27				NEG
1879		NEG	0.20				NEG
1880		NEG	0.59				NEG
1881	p24	NEG	0.78				NEG
1882		NEG	0.30				NEG
1883	p19	NEG	0.41				NEG
1884		NEG	0.43				NEG
1885		NEG	0.26				NEG
1886		NEG	0.26				NEG
1887		NEG	0.53				NEG
1888		NEG	0.66				NEG
1889		NEG	0.35				NEG
1890		NEG	0.60				NEG
1891		NEG	0.39				NEG
1892	p24	NEG	0.32				NEG
1893		NEG	0.30				NEG
1894		NEG	0.53				NEG
1895		NEG	0.26				NEG
1896	p24	NEG	0.93				NEG
1897		NEG	0.31				NEG
1898	p24	NEG	0.82				NEG
1899		NEG	0.28				NEG
1900		NEG	0.21				NEG
1901		NEG	0.32				NEG
1906		NEG	0.19				NEG
1907		NEG	0.36				NEG
1908		NEG	0.21				NEG
1909		NEG	0.24				NEG
1910		NEG	0.28				NEG
1911		NEG	0.14				NEG
1912		NEG	0.66				NEG
1914		NEG	0.36				NEG
1915		NEG	0.60				NEG
1916		NEG	0.61				NEG
1917		NEG	0.61				NEG
1918		NEG	0.78				NEG
1919		NEG	0.27				NEG
1920		NEG	1.05/1.32/1.30	Non-Reactive	p24		NEG
1921		NEG	0.25				NEG
1922		NEG	0.67				NEG
1923		NEG	0.27				NEG
1924		NEG	0.28				NEG
1925		NEG	0.20				NEG
1926		NEG	0.28				NEG
1927		NEG	0.23				NEG
1928		NEG	0.26				NEG
1929		NEG	0.33				NEG
1930		NEG	0.26				NEG
1931		NEG	0.32				NEG
1932		NEG	0.87				NEG
1933		NEG	0.21				NEG
1934	p19	NEG	0.26				NEG
1935		NEG	0.26				NEG
1936		NEG	0.29				NEG

Table 25: EIA Negative Population HTLV Blot 2.4 / CDPHL Algorithm Concordant Results

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1937		NEG	0.27				NEG
1938		NEG	0.33				NEG
1939		NEG	0.24				NEG
1940		NEG	0.56				NEG
1941		NEG	0.43				NEG
1942		NEG	0.36				NEG
1944		NEG	0.40				NEG
1946		NEG	0.23				NEG
1947		NEG	0.74				NEG
1948		NEG	0.21				NEG
1949		NEG	0.56				NEG
1950		NEG	0.54				NEG
1952		NEG	0.27				NEG
1955		NEG	0.48				NEG
1956		NEG	0.26				NEG
1957	p19	NEG	10.00/7.59/7.77	Non-reactive	Non-reactive		NEG
1958		NEG	1.05/0.56/0.58				NEG
1961		NEG	0.43				NEG
1962		NEG	0.39				NEG
1963		NEG	0.31				NEG
1964		NEG	1.99/2.38/2.31	Non-reactive	Non-reactive		NEG
1965		NEG	0.31				NEG
1966		NEG	0.34				NEG
1968		NEG	0.24				NEG
1969		NEG	0.46				NEG
1970		NEG	0.36				NEG
1971		NEG	0.45				NEG
1972		NEG	0.29				NEG
1973		NEG	0.34				NEG
1974		NEG	0.33				NEG
1975		NEG	0.30				NEG
1977		NEG	0.22				NEG
1978		NEG	0.36				NEG
1979		NEG	1.29/0.96/1.03	Non-reactive	Non-reactive		NEG
1980	p24	NEG	0.32				NEG
1981		NEG	0.31				NEG
1982		NEG	0.30				NEG
1983		NEG	0.40				NEG
1984		NEG	0.33				NEG
1985		NEG	0.47				NEG
1986		NEG	0.28				NEG
1987		NEG	1.48/1.11/1.21	Non-reactive	Non-reactive		NEG
1988		NEG	0.33				NEG
1989		NEG	0.33				NEG
1990		NEG	1.24/0.91/1.10	Non-reactive	P21E	Non-reactive	NEG
1991		NEG	0.53				NEG
1992		NEG	0.37				NEG
1993		NEG	0.21				NEG
1994		NEG	0.29				NEG
1995		NEG	0.42				NEG
1996		NEG	0.46				NEG
1997		NEG	0.31				NEG
1998		NEG	0.29				NEG
1999		NEG	0.36				NEG
2000		NEG	0.22				NEG
2002		NEG	0.49				NEG

Table 25: EIA Negative Population HTLV Blot 2.4 / CDPHL Algorithm Concordant Results

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2003		NEG	0.34				NEG
2004	p24	NEG	0.43				NEG
2005		NEG	0.33				NEG
2006		NEG	0.21				NEG
2007	p24	NEG	1.83/2.00/2.00	Non-reactive	P21E	Non-reactive	NEG
2008		NEG	0.44				NEG
2009		NEG	2.52/2.43/2.47	Non-reactive	Non-reactive		NEG
2010		NEG	0.33				NEG
2011		NEG	0.44				NEG
2012		NEG	0.47				NEG
2013		NEG	0.39				NEG

Of these 169 samples, 14 were repeatedly reactive on the CDPHL HTLV in-house ELISA, leading to additional supplemental testing. Comparatively, all but two of these reactive ELISA samples were clean negatives as determined by the absence of HTLV bands present on the HTLV Blot 2.4 assay. Of the two RR samples that showed HTLV specific protein reactivity on the HTLV Blot 2.4, one showed reactivity to the p19 band (sample 1957) and the other to the p24 band (sample 2007).

d) HTLV Screening Reactive Population

The comparison between the HTLV Blot 2.4 assay and the CDPHL Algorithm on percent positive agreement was evaluated by testing 200 HTLV screening reactive specimens, herein EIA RR population. These samples were qualified as testing previously repeatedly reactive using a licensed HTLV screening assay, followed by a second RR result from a non-licensed screening assay, the CDPHL ELISA. These specimens were obtained from the well-characterized ARC repository and were aliquots of plasma units that had screened RR on both the licensed Abbott PRISM HTLV-I/HTLV-II ChLIA assay and were unconfirmed by an additional HTLV screening assay or any further HTLV supplemental testing. The 200 EIA RR samples were divided among the ARC Charlotte NTL, LABS, Inc., and CDPHL for evaluation on the HTLV Blot 2.4; testing was performed using three product lots. All specimens were tested by HTLV Blot 2.4 assay and the CDPHL Algorithm, and all individual HTLV Blot 2.4 results were compared to that of the CDPHL Algorithm. The objective of testing an unconfirmed repeatedly reactive population was to assess the performance of the HTLV Blot 2.4 in detecting infected individuals over false positive samples; therefore, the results indicated will be based on a percent positive agreement with the TOR considered as positive.

Of the 200 samples tested in the repeatedly reactive population, the HTLV Blot 2.4 identified 8 as positive based on the HTLV Blot 2.4 interpretation criteria; 91 samples were identified as indeterminate, and 102 as negative, based on the same criteria. The CDPHL Algorithm identified 3 samples as indeterminate and 197 as negative; no positive samples were identified by the CDPHL Algorithm. The percent positive agreement of the HTLV Blot 2.4 with the TOR considered positive, calculated with indeterminate results as negative, is reported as 4.0 % with a 95% CI as 1.74% to 7.73% as indicated in **Table 26.**

Table 26: EIA RR Population Percent Positive Agreement

		CDPHL Algorithm			
		Positive	***Indeterminate***	***Negative***	***Total***
MP HTLV Blot 2.4	***Positive***	0	0	7	8 (4.0%)
	Indeterminate	0	0	91	90 (45.0%)
	Negative	0	3	99	102 (51.0%)
	Total	0 (0.0%)	3 (1.5%)	197 (98.5%)	200

1) HTLV Blot 2.4 Positive Samples

In total, the HTLV Blot 2.4 identified eight samples with HTLV seropositivity. A breakdown of the individual results of each positive sample, along with the corresponding CDPHL HTLV Algorithm result is indicated in **Table 27.**

Table 27: EIA RR Population HTLV Blot 2.4 Positive Samples

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2145	GD21, p24, rgp46-II, rgp46-I	HTLV-II	1.02/1.10/1.21	Non-reactive	Non-reactive		NEG
2148	GD21, p19, p24, rgp46-I	HTLV-I	1.03/0.95/1.03	Non-reactive	p19		NEG
2153	GD21, p19, p24, rgp46-I	HTLV-I	0.65				NEG
2155	GD21, p19, p24, rgp46-II, rgp46-I	HTLV-I/II	4.36/2.97/2.43	Non-reactive	Non-reactive		NEG
2159	GD21, p19, p24, p36	HTLV-I	0.86				NEG
2170	GD21, p19, p24, p26, p28, p32, p36, gp46, p53	HTLV-I	4.92/6.26/6.70	Non-reactive	p24, p21E		NEG
2176	*GD21, p19, rgp46-II, rgp46-I*	*HTLV-I*[a]	*0.61*				*NEG*
2189	GD21, p24, rgp46-II	HTLV-II	0.49				NEG

[a] This sample was incorrectly resulted as Indeterminate. A band pattern of GD21, p19 and rgp46-I should result in a HTLV-I interpretation.

The frequency of HTLV band patterns seen in these positive samples is indicated in **Table 28.**

Table 28: EIA RR Population HTLV Blot 2.4 HTLV Positive Band Patterns (n = 8)

HTLV Blot 2.4 Band Pattern	Frequency
GD21, p19, p24, rgp46-I	2 (25.0%)
GD21, p24, rgp46-II	1 (12.5%)
GD21, p24, rgp46-II, rgp46-I	1 (12.5%)
GD21, p19, p24, rgp46-II, rgp46-I	1 (12.5%)
GD21, p19, p24,p36	1 (12.5%)
GD21, p19, p24, p26, p28, p32, p36, gp46, p53	1 (12.5%)
GD21, p19, rgp46-II, rgp46-I	1 (12.5%)

The HTLV Blot 2.4 reported 8 out of 200 samples as positive in the RR population, resulting in a seropositive rate of 4%. These results are more acceptable for the population size than the CDPHL results. In 2011, the CDPHL tested 2389 non-diluted samples using their HTLV algorithm; of these 2389 undiluted samples, 311 were positive for a seropositivity rate of 13%. Extrapolating this seropositivity rate to the existing RR population, one would expect approximately 26 samples to be seropositive. The HTLV Blot 2.4 reactivity rate of 4% is below the extrapolated value (13%) but could be due to the dilution of the samples. Therefore, it is noted that the HTLV Blot 2.4 is far more sensitive in detecting positive samples than the CDPHL Algorithm.

2) EIA RR Population HTLV Blot 2.4 Negative Samples

Of the 200 samples enrolled in the RR population, the HTLV Blot 2.4 resulted 102 as negative based on the interpretation criteria. A sample is negative when no HTLV specific bands are present, or when non-critical bands are present in a pattern that meets the criteria for seronegative. Common seronegative band patterns and their frequencies are indicated in **Table 29.**

Table 29: EIA RR Population HTLV Blot 2.4 HTLV Negative Band Patterns (n = 99)

HTLV Blot 2.4 Band Pattern	Frequency
Clean (no bands)	61 (61.6%)
p19, p26, p28, p32, p36 (HGIP)	18 (18.2%)
p19	11 (11.1%)
p19, p26, p28, p32, p26, p53 (HGIP)	3 (3.0%)
p24	3 (3.0%)
p19, p26, p28, p36 (HGIP)	1 (1.0%)
p19, p36	1 (1.0%)
p19, p26	1 (1.0%)

11. INTERPRETATION CRITERIA VALIDITY

a) Specificity of GD21 as Compared to p21e

GD21 is a truncated portion of p21e, shown to be non-immunoreactive to sera from individuals with documented reactivity to p21e but PCR negative. The HTLV Blot 2.4 uses GD21 instead of p21e to increase product specificity.

The specificity of GD21 versus p21e was assessed for the EIA negative and EIA RR populations; sample results showing specific p21e reactivity with the CDPHL HTLV Western Blot are indicated in **Table 30** below.

Table 30: p21e Reactive Samples

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL WB*	*CDPHL Western Blot Final Results*
2079		NEG	p21e	IND
1990		NEG	p21e	IND
2007	p24	NEG	p21e	IND
2088		NEG	p21e	IND
2109	GD21, p53	IND	p21e	IND
2281	p19, p26, p28, p36	NEG	p19, p24, p36, p21e, p53	Reactive
2307	p19, p26, p28, p32, p36	NEG	p19, p24 p21e	Reactive
2202	rgp4-II	IND	p21e	IND
2213	p19, p26, p28, p32, p36	NEG	p19, p28, p21e, p53	Reactive

Nine samples showed p21e reactivity using the CDPHL Western Blot. Per the interpretation criteria of the CDPHL Western Blot, samples with p21e activity alone are resulted as indeterminate, and those with p21e and p19 and/or p24 are considered HTLV-I reactive. Therefore, of the nine samples with p21e reactivity, three were resulted as HTLV-I reactive and six as indeterminate. Conversely, the HTLV Blot 2.4 identified seven as seronegative and two as indeterminate. Only one of the HTLV Blot 2.4 IND samples was indeterminate due to the presence of GD21; the other showed immunoreactivity to rgp46-II. All three samples resulted as HTLV-I reactive by the CDPHL HTLV Western Blot were negative by the criteria of the HTLV Blot 2.4. The final results for the HTLV Blot 2.4 and the CDPHL Western Blot are indicated in **Table 31.** If indeterminate samples are considered false positive, then substitution of the p21e antigen with GD21 resulted in a 78.2% decrease in false positives due to non-specific immunoreactivity.

Table 31: GD21 / p21e Specificity

		CDPHL Algorithm		
		Positive	***Negative***	***Total***
MP HTLV	***Positive***	2	0	2 (22.2%)
Blot 2.4	***Negative***	7	0	7 (77.8%)
	Total	9 (100.0%)	0	9

b) p19, p24 Relative Intensity as HTLV type Indicator

In the absence of a type specific recombinant protein (i.e. rgp46-II or rgp46-I), HTLV type discrimination can be made based on the relative intensities of p19 and p24. In HTLV-I type 1 specimens, the relative intensity of p19 is greater than or equal to that of HTLV-II; conversely, in HTLV type 2 specimens, the relative intensity of p24 is greater than that of p19[55].

The relative intensities of p19 and p24 were assessed for all samples (n = 105) differentiated as HTLV-I (n = 82) or HTLV-II (n = 103) in the known positive population. The results are indicated in **Table 32** below.

Table 32: HTLV Type Differentiation by p19/p24 Relative Intensity

HTLV Type Differentiation	p19 ≥ p24	p24 > p19
HTLV-I	82	0
HTLV-II	2	101

Based on the results above, the relative intensities of the *gag* proteins were correct for 82 (100.0%) of the HTLV-I specimens and 101 (98.1%) of HTLV-II specimens. The two HTLV-II specimens, the relative intensities of the p19 and p24 bands, and the final interpretations of the specimens by both the HTLV Blot 2.4 and the CDPHL HTLV Algorithm are indicated in **Table 33.**

Table 33: p19/p24 Relative Intensity Discordant Samples

HTLV Blot 2.4 Band Pattern	p19 intensity	p24 intensity	HTLV Blot 2.4 Result	CDPHL Final Result
GD21, p19, p24, rgp46-II[a]	1+	+/-	HTLV-II	HTLV-II
GD21, p19, p24, p26, p28, p32, p36, rgp46-II[b]	2+	1+	HTLV-II	HTLV-II

[a]Source: Clinical sample 1740
[b]Source: Clincal sample 1801

As indicated above, both the HTLV Blot 2.4 and CDPHL HTLV Algorithm results interpreted the specimen as HTLV-II. Although the intensities of the *gag* proteins varied, the specimens were resulted by the presence of a type specific recombinant. Use of the relative intensity of the *gag* proteins is only

used in cases where a type specific recombinant is not present, so only cases that fit this criteria was further evaluated. **Table 34** lists specimens from the known positive population that lacked the presence of a type specific recombinant, and in which HTLV viral type discrimination depended on the relative intensities of the p19 and p24 *gag* proteins. A final result given by the HTLV Blot 2.4, along with the CDPHL final result as well as the IFA titer results used for viral type differentiation.

Table 34: Viral Type Discrimination with Absence of Type Specific Recombinant

Specimen ID	GD21 Intensity	P19 Intensity	P24 Intensity	HTLV Blot 2.4 Result	CDPHL IFA	CDPHL Final Result
1863	3+	3+	+/-	HTLV-I	HTLV-I: (1:256) HTLV-II: (1:64)	HTLV-I
1866	2+	3+	1+	HTLV-I	HTLV-I: (1:8) HTLV-II: (1:32)	HTLV-II
1805	1+	+/-	1+	HTLV-II	HTLV-I: (1:16) HTLV-II: (1:256)	HTLV-II
1718	3+	3+	3+	HTLV-I	HTLV-I: (1:256) HTLV-II: (1:64)	HTLV-I
1759	2+	2+	1+	HTLV-I	HTLV-I: (1:64) HTLV-II: (1:16)	HTLV-I

In the known positive population, five specimens were confirmed and differentiated by the HTLV without the presence of a type specific recombinant antigen. Four out of five of the HTLV Blot 2.4 type discrimination results were concordant with the CDPHL final interpretations. Review of the nitrocellulose strips for these five specimens ***(Figure 13)*** confirmed that the operator had correctly interpreted and scored the bands present according to the HTLV Blot 2.4 interpretative criteria.

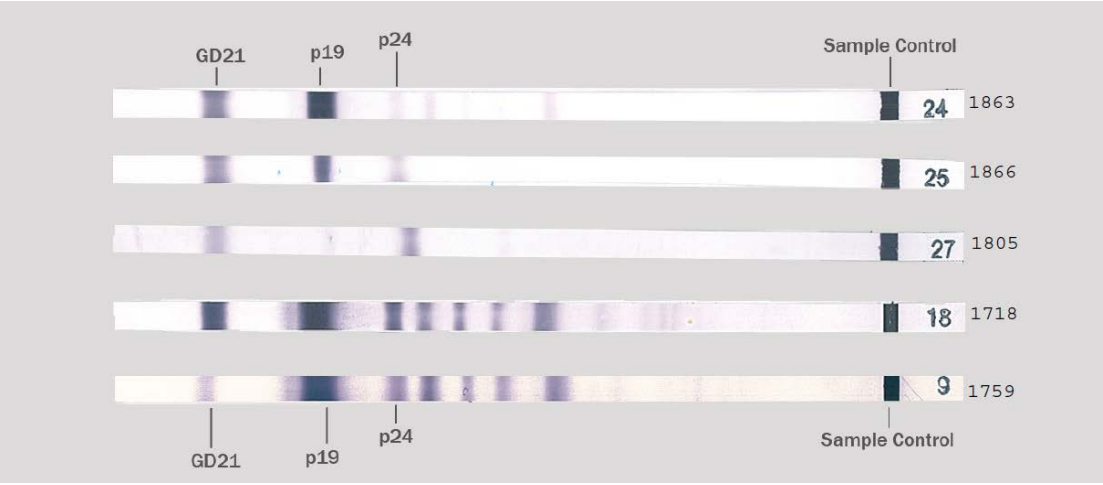


Figure 13: HTLV Type Discrimination Using p19 and p24 Relative Intensity

c) HGIP/Gag Proteins as Seronegative

Donors with specimens showing reactivity to specific profiles of *gag* proteins previously considered indeterminate have been indicated through follow-up activities such as donor follow-up, clinical diagnosis, and PCR testing to be uninfected (i.e. seronegative) with the HTLV virus. These profiles include the HTLV Gag Indeterminate Profile, traditionally termed HGIP, which consists of a combination of *gag* proteins p19, 26, p28, p32, p36 and p53 without the presence of p24. Additionally, *gag* proteins in any combination, again with the presence of p24, are consisted seronegative. Finally, any single *gag* protein, including p24, may be considered seronegative.

The HTLV Blot 2.4 results from all three clinical populations were assessed for adherence to the seronegative aspect of these *gag* profiles as per the HTLV Blot 2.4 interpretative criteria. In the known positive population, those specimens previously confirmed as reactive to antibodies to HTLV-I/II, no specimens were identified as containing the *gag* profiles listed above. The one specimen in the known positive population identified as negative by the HTLV Blot 2.4 was a clean negative; the negative result was due to the dilution of the specimen below the detectable level of the assay.

Of the 200 EIA negative specimens, twelve were identified as containing a single *gag* protein, p19 or p24; eight HTLV Blot 2.4 negative specimens (4.7%) showed reactivity to p24 alone and four (2.4%) showed reactivity to p19 alone. Of the 200 EIA RR specimens, 99 were negative by the criteria of the HTLV Blot 2.4, with a significant portion (n = 21) showing traditional HGIP as indicated earlier in **Table 29.** Another 17 specimens reacted to either a single *gag* protein (i.e. p19 or p24) or contained a combination of *gag* proteins. All corresponding CDPHL results indicated negative.

12. ENSURING END USER COMPETENCY

Accurate identification and interpretation of the HTLV bands present on a specimen test strip is an integral aspect of the HTLV Blot 2.4 kit. MP Biomedicals Asia Pacific offers a combination of labeling and education to minimize the likelihood of erroneous results due to misinterpretation of the viral bands by an end user.

a) Labeling

The labeling associated with the MP Diagnostics HTLV Blot 2.4 includes a package insert (PI), also termed Instructions for Use (IFU), as well as several other labeling items designed to assist an end user in identifying and interpreting HTLV viral bands. The HTLV Blot 2.4 package insert contains instruction information on the following: reagent preparation for varying batch sizes; the assay procedure, including a summary of assay protocols; assay validity; and interpretation criteria that defines the results of test specimens based on the presence or absence of bands. Other labeling items were briefly described in **Section 8b: Assay Principle,** and include a Protein Finder¸ an Intensity Finder, and a Report Sheet.

The Protein Finder ***(Figure 02)*** is a separate, lot specific, labeling item included with each HTLV Blot 2.4 kit, and consists of an HTLV viral band schematic with each HTLV viral band identified, positioned next to a nitrocellulose strip assayed using a control sample strongly immunoreactive to anti-HTLV-I. The nitrocellulose strip used in the Protein Finder is obtained from the same member as the test strips in the kit; therefore, any variations in the distance of protein migration during nitrocellulose strip production are minimized. In the IFU, the end user is instructed to use the lot specific Protein Finder to identify each viral band on each control sample (anti-HTLV-I and anti-HTLV-II) and then use the control samples to identify the bands on each test sample. Overall, use of a lot specific Protein Finder and the control strips mitigates the risk of misidentification of a viral band as the placement of each band for each membrane is specifically identified; therefore, lot-to-to variation created by different nitrocellulose membranes is minimized.

The Intensity Finder ***(Figure 03)*** is a controlled document that denotes the approximate intensities of different band intensities on an increasing scale. The HTLV Blot 2.4 assay can be scored and the bands patterns interpreted based on the presence or absence of specific HTLV bands, or on the intensity of specific HTLV bands present. In some cases as previously described, such as a test specimen presenting with recombinant GD21, p19 and p24 without the presence of a type specific recombinant, HTLV viral type discrimination is dependent upon the intensity of key bands. Additionally, the HTLV Blot 2.4 interprets any band present, regardless of intensity, and identification of weak bands may be challenging. Therefore, the Intensity Finder contains schematic intensities ranging from +/- to 3+ to aid in scoring

bands on an intensity scale. The end-user would line up the Intensity Finder with the band in question, and compare the viral band on the test strip with that on the Intensity Finder.

Finally, the HTLV Blot 2.4 contains a Report Sheet ***(Figure 04).*** This item allows the end user to paste developed strips and easily score for the presence / absence of bands, or the intensity of bands present. Additionally, this report sheet is a convenient way for end-users to store and organized strips for archival purposes.

b) MP Biomedicals Educational Programs

In addition to the labeling that accompanies the HTLV Blot 2.4, MP Biomedicals is proposing an educational program to assist end-users specifically with viral band interpretation. This educational program will consist of the following items:

- Instructional video illustrating the assay procedural steps with emphasis on use of the protein finder and controls for result interpretation;
- Instructional interpretation guide focused on result interpretation designed for repeated use;
- Certified online training program, which will include general assay comprehension and competency testing in result interpretation using test sources derived from challenging clinical samples;
- Educational and interactive webinars as appropriate;
- Location based training as appropriate.

13. RISK / BENEFIT ANALYSIS

The expected benefit from commercialization of a licensed HTLV confirmatory assay is as follows:

- Correct, reliable interpretation of RR specimens for the purpose of donor counseling and treatment;
- Unnecessary donor loss (under donor reentry) due to diminished specificity of HTLV screening assays.

The results of the U.S. Clinical Trial, including but not limited to the screening reactive population (i.e. EIA RR cohort), strongly suggest that both benefits will be actualized from licensure of the HTLV Blot 2.4. This RR population was the most representative of the blood donor population in that the specimens were repeat reactive by one licensed HTLV screening assay, one unlicensed HTLV screening assay, but unconfirmed.

Of the 200 specimens in the RR population, 102 (51.0%) tested negative by the criteria of the HTLV Blot 2.4. These negative results were supported by the reference test results, and are therefore assumed to be from individuals uninfected with HTLV-I/II. A negative supplemental assay result would provide reassurance to the donor of their uninfected state. Of the 200 specimens in the RR population, 8 (4.0%) were determined to be seropositive by the criteria of the HTLV Blot 2.4; of the eight positive, five were typed as HTLV-I, two as HTLV-II, and one as HTLV-I/II. The CDPHL HTLV Supplemental Algorithm did not interpret any of these specimens as positive, although prevalence rates, along with previous seropositivity rates, would indicate otherwise. Therefore, the MP Diagnostics HTLV Blot 2.4 is more sensitive in detecting lower level positives.

Comparatively, no samples were identified as positive in the EIA HTLV Screening Non-reactive Population, demonstrating that the HTLV Blot 2.4 does not misidentify sample reactivity, and the risk of false positives is low. The sensitivity of the HTLV Blot 2.4 in the Known Positive Population was 97.50%. Overall, the HTLV Blot 2.4 demonstrated appropriate performance required for an HTLV supplemental assay.

14. CONCLUSIONS

Currently, there exists an unmet need for an HTLV supplemental assay, capable of confirming HTLV infection and discriminating between HTLV viral band types. The MP Diagnostics HTLV Blot 2.4 Western blot assay is a supplemental assay that uses a combination of specialized recombinant antigens and native viral proteins to confirm and differentiate HTLV infection. The HTLV Blot 2.4 uses a combination of HTLV-I/II genetically engineered proteins (recombinant proteins) and HTLV-I viral proteins derived from native, inactivated viral particles (viral lysate). The differentiation between HTLV-I and HTLV-II is accomplished with rgp46-I (MTA-1), an unique HTLV-I envelope recombinant protein, and rgp46-II (K-55), a unique HTLV-II envelope recombinant protein. Both proteins are derived from the central region of the external glycoprotein, gp46, of the respective HTLV-I and HTLV-II viral types. GD21, a common yet specific HTLV-I and HTLV-II epitope envelope recombinant protein (rgp21), is also used to enhance the sensitivity of envelope antibody detection. The antigenicity exhibited by these proteins is either common to HTLV-I and HTLV-II antibodies, or type specific to one of the two viruses to allow confirmation and discrimination in a single assay. Additionally, the presence of native viral proteins p19 and p24 allow for confirmation and discrimination in cases of non-immunoreactivity to a type specific recombinant antigen.

The performance of the HTLV Blot 2.4 was established during clinical studies designed to evaluated the reproducibility, sensitivity and validity of the assay along with the defined interpretation criteria. The HTLV Blot 2.4 demonstrated reproducibility within assay, within operator, within lot, within site, and within panel member. The sensitivity of the HTLV Blot 2.4, as tested within a Known Positive Population, was determined to 97.50%, higher than that of the non-reference standard. In the EIA HTLV screening negative population, the HTLV Blot 2.4 did not identify any of the samples as positive, validating the interpretative criteria. In the EIA HTLV screening reactive population, the HTLV Blot 2.4 identified eight samples as positive, demonstrating increased sensitivity over the non-reference standard. Additionally, use of GD21 as a recombinant increases specificity, and further discrimination is available through the use of p19 and p24.

In conclusion, the MP Diagnostics HTLV Blot 2.4 is an applicable product to fulfill the need for an HTLV confirmatory assay.

15. BIBLIOGRAPHY

1. Cann AJ, Chen ISY. Human T-cell leukemia virus types I and II. In Fields BN and Knipe DM (eds). Virology. 2nd ed. New York: Raven Press, Ltd.; 1990:1501-27.
2. Centers for Disease Control. Update: Serologic testing for human T-lymphotropic virus type I - United States, 1989 and 1990. MMWR 1992;41:259-63.
3. Chen YM, Lee TH, Wiktor SZ, et al. Type-specific antigens for serological discrimination of HTLV-I and HTLV-II infection. Lancet 1990;336:1153-5.
4. Eble BE, Busch MP, Guiltinan AM, Khayam-Bashi H, Murphy EL. Determination of human T lymphotropic virus type by polymerase chain reaction and correlation with risk factors in northern California blood donors. J Infect Dis 1993;167(4):954-7.
5. Wattel E, Mariotti M, Agis F, et al. Quantification of HTLV-1 proviral copy number in peripheral blood of symptomless carriers from the French West Indies. J Acquir Immune Defic Syndr 1992;5(9):943-6.
6. Cimarelli A, Duclos CA, Gessain A, et al. Quantification of HTLV-II proviral copies by competitive polymerase chain reaction in peripheral blood mononuclear cells of Italian injecting drug users, central Africans, and Amerindians. J Acquir Immune Defic Syndr Hum Retrovirol 1995;10(2):198-204.
7. Manns A, Blattner WA. The epidemiology of the human T-cell lymphotrophic virus type I and type II: etiologic role in human disease. Transfusion 1991;31(1):67-75.
8. Murphy EL, Watanabe K, Nass CC, Ownby H, Williams A, Nemo G. Evidence among blood donors for a 30-year-old epidemic of human T lymphotropic virus type II infection in the United States. J Infect Dis 1999;180(6):1777-83.
9. Schreiber GB, Murphy EL, Horton JA, et al. Risk factors for human T-cell lymphotropic virus types I and II (HTLV-I and -II) in blood donors: the Retrovirus Epidemiology Donor Study. NHLBI Retrovirus Epidemiology Donor Study. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14(3):263-71.
10. de The G, Bomford R. An HTLV-I vaccine: why, how, for whom? AIDS Res Hum Retroviruses 1993;9(5):381-6.
11. Hjelle B, Khabbaz RF, Conway GA, North C, Green D, Kaplan JE. Prevalence of human T cell lymphotropic virus type II in American Indian populations of the southwestern United States. Am J Trop Med Hyg 1994;51(1):11-5.
12. Reeves WC, Cutler JR, Gracia F, et al. Human T cell lymphotropic virus infection in Guaymi Indians from Panama. Am J Trop Med Hyg 1990;43(4):410-8.
13. Maloney EM, Biggar RJ, Neel JV, et al. Endemic human T cell lymphotropic virus type II infection among isolated Brazilain Amerindians. J Infect Dis 1992;166:100-7.
14. Fujiyoshi T, Li HC, Lou H, et al. Characteristic distribution of HTLV type I and HTLV type II carriers among native ethnic groups in South America. AIDS Res Hum Retroviruses 1999;15(14):1235-9.
15. Zhu SW, Horal P, Furuta Y, Zagaany G, Vahlne A. HTLV-II infection in Mongolia. Abstract 2. AIDS Res Hum Retroviruses 1994;10:443.
16. Goubau P, Liu HF, de Lange GG, Vandamme AM, Desmyter J. HTLV-II seroprevalence in Pygmies across Africa since 1970. AIDS Research and Human Retroviruses 1993;9:709-13.
17. Gessain A, de The G. What is the situation of human T cell lymphotropic virus type II (HTLV-II) in Africa? Origin and dissemination of genomic subtypes. J Acquir Immune Defic Syndr Hum Retrovirol 1996;13 Suppl 1:S228-35.
18. Slattery JP, Franchini G, Gessain A. Genomic evolution, patterns of global dissemination, and interspecies transmission of human and simian T-cell leukemia/lymphotropic viruses. Genome Res 1999;9(6):525-40.

19. Vandamme AM, Salemi M, Van Brussel M, et al. African origin of human T-lymphotropic virus type 2 (HTLV-2) supported by a potential new HTLV-2d subtype in Congolese Bambuti Efe Pygmies. J Virol 1998;72(5):4327-40.

20. Vandamme AM, Bertazzoni U, Salemi M. Evolutionary strategies of human T-cell lymphotropic virus type II. Gene 2000;261(1):171-80.

21. Robert-Guroff M, Weiss SH, Giron JA, et al. Prevalence of antibodies to HTLV-I, -II, and -III in intravenous drug abusers from an AIDS endemic region. Jama 1986;255(22):3133-7.

22. Lee H, Swanson P, Shorty VS, Zack JA, Rosenblatt JD, Chen IS. High rate of HTLV-II infection in seropositive IV drug abusers in New Orleans. Science 1989;244:471-5.

23. Feigal E, Murphy E, Vranizan K, et al. Human T cell lymphotropic virus type I and II in intravenous drug users in San Francisco: Risk factors associated with seropositivity. J Infect Dis 1991;164:36-42.

24. Ehrlich GD, Glaser JB, LaVigne K, et al. Prevalence of human T-cell leukemia/lymphoma virus (HTLV) type II infection among high-risk individuals: type-specific identification of HTLVs by polymerase chain reaction. Blood 1989;74(5):1658-64.

25. Khabbaz RF, Onorato IM, Cannon RO, et al. Seroprevalence of HTLV-1 and HTLV-2 among intravenous drug users and persons in clinics for sexually transmitted diseases. N Engl J Med 1992;326(6):375-80.

26. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc Natl Acad Sci U S A 1982;79(6):2031-5.

27. Leite AC, Mendonca GA, Serpa MJ, Nascimento OJ, Araujo AQ. Neurological manifestations in HTLV-I-infected blood donors. J Neurol Sci 2003;214(1-2):49-56.

28. Shimazaki R, Ueyama H, Mori T, et al. Chronic sensory neuronopathy associated with human T-cell lymphotropic virus type I infection. J Neurol Sci 2002;194(1):55-8.

29. Leite AC, Silva MT, Alamy AH, et al. Peripheral neuropathy in HTLV-I infected individuals without tropical spastic paraparesis/HTLV-I-associated myelopathy. J Neurol 2004;251(7):877-81.

30. Morgan DJ, Caskey MF, Abbehusen C, et al. Brain magnetic resonance imaging white matter lesions are frequent in HTLV-I carriers and do not discriminate from HAM/TSP. AIDS Res Hum Retroviruses 2007;23(12):1499-504.

31. Murphy EL, Wang B, Sacher RA, et al. Respiratory and urinary tract infections, arthritis, and asthma associated with HTLV-I and HTLV-II infection. Emerg Infect Dis 2004;10(1):109-116.

32. Rocha PN, Rehem AP, Santana JF, et al. The cause of urinary symptoms among Human T Lymphotropic Virus Type I (HLTV-I) infected patients: a cross sectional study. BMC Infect Dis 2007;7:15

33. Castro NM, Rodrigues W, Jr., Freitas DM, Muniz A, Oliveira P, Carvalho EM. Urinary symptoms associated with human T-cell lymphotropic virus type I infection: evidence of urinary manifestations in large group of HTLV-I carriers. Urology 2007;69(5):813-8.

34. Caskey MF, Morgan DJ, Porto AF, et al. Clinical manifestations associated with HTLV type I infection: a cross-sectional study. AIDS Res Hum Retroviruses 2007;23(3):365-71.

35. Castro N, Oliveira P, Freitas D, Rodrigues W, Muniz A, Carvalho E. Erectile dysfunction and HTLV-I infection: a silent problem. International journal of impotence research 2005;17(4):364-9.

36. Silva MT, Leite AC, Alamy AH, Chimelli L, Andrada-Serpa MJ, Araujo AQ. ALS syndrome in HTLV-I infection. Neurology 2005;65(8):1332-3.

37. Silva MT, Mattos P, Alfano A, Araujo AQ. Neuropsychological assessment in HTLV-1 infection: a comparative study among TSP/HAM, asymptomatic carriers, and healthy controls. Journal of neurology, neurosurgery, and psychiatry 2003;74(8):1085-9.

38. Sawa H, Nagashima T, Nagashima K, et al. Clinicopathological and virological analyses of familial human T-lymphotropic virus type I--associated polyneuropathy. J Neurovirol 2005;11(2):199-207.

39. Arakawa K, Umezaki H, Noda S, Itoh H. Chronic polyradiculoneuropathy associated with human T-cell lymphotropic virus type I infection. Journal of neurology, neurosurgery, and psychiatry 1990;53(4):358-9.

40. Douen AG, Pringle CE, Guberman A. Human T-cell lymphotropic virus type 1 myositis, peripheral neuropathy, and cerebral white matter lesions in the absence of spastic paraparesis. Arch Neurol 1997;54(7):896-900.

41. Gebretsadik T, Murphy EL. Counseling and medical evaluation of HTLV-I and -II infected patients. In Volberding P and Jacobson M (eds.). AIDS Clinical Review. New York: Marcel Dekker; 1993.

42. Murphy EL, Glynn SA, Fridey J, et al. Increased incidence of infectious diseases during prospective follow-up of human T-lymphotropic virus type II- and I-infected blood donors. Retrovirus Epidemiology Donor Study. Arch Intern Med 1999;159(13):1485-91.

43. Rosenblatt JD, Giorgi JV, Golde DW, et al. Integrated human T-cell leukemia virus II genome in CD8 + T cells from a patient with "atypical" hairy cell leukemia: evidence for distinct T and B cell lymphoproliferative disorders. Blood 1988;71(2):363-9.

44. Harrington WJ, Jr., Sheremata W, Hjelle B, et al. Spastic ataxia associated with human T-cell lymphotropic virus type II infection. Ann Neurol 1993;33(4):411-4.

45. Sheremata WA, Harrington WJ, Jr., Bradshaw PA, et al. Association of '(tropical) ataxic neuropathy' with HTLV-II. Virus Res 1993;29(1):71-7.

46. Black FL, Biggar RJ, Lal RB, Gabbai AA, Filho JP. Twenty-five years of HTLV type II follow-up with a possible case of tropical spastic paraparesis in the Kayapo, a Brazilian Indian tribe. AIDS Res Hum Retroviruses 1996;12(17):1623-7.

47. Jacobson S, Lehky T, Nishimura M, Robinson S, McFarlin DE, Dhib-Jalbut S. Isolation of HTLV-II from a patient with chronic, progressive neurological disease clinically indistinguishable from HTLV-I-associated myelopathy/tropical spastic paraparesis. Ann Neurol 1993;33(4):392-6.

48. Lehky TJ, Flerlage N, Katz D, et al. Human T-cell lymphotropic virus type II-associated myelopathy: clinical and immunologic profiles. Ann Neurol 1996;40(5):714-23.

49. Biglione MM, Pizarro M, Salomon HE, Berria MI. A possible case of myelopathy/tropical spastic paraparesis in an Argentinian woman with human T lymphocyte virus type II. Clin Infect Dis 2003;37(3):456-8.

50. Toro C, Blanco F, Garcia-Gasco P, et al. Human T lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis in an HIV-positive patient coinfected with human T lymphotropic virus type 2 following initiation of antiretroviral therapy. Clin Infect Dis 2007;45(9):e118-20.

51. Araujo A, Hall WW. Human T-lymphotropic virus type II and neurological disease. Ann Neurol 2004;56(1):10-9.

52. Guidance for Industry: Donor Screening for Antibodies to HTLV-II, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research (CBER), August 1997.

53. U.S. Patent 5,643,714

54. U.S. Patent 6,110,662

55.Wiktor S.Z., Alexander S.S., Shaw G.M., Weiss S.H., Murphy E.L., Wilks R.J., Shortly V.J., Hanchard B., and Blatther W.A. Distinguishing between HTLV-I and HTLV-II by Western Blot. Lancet 1990; 335: 533.

56. Public Health Service Working Group. Licensure of screening test for antibody to human T-lymphotropic virus type I. Morb. Mortal. Wkly Rep. 1988: 37:736–747

57. Virus Information Exchange Newsletter, Vol 7(2), June 1990, 54-55

58. Centers for Disease Control and Prevention and the USPHS Working Group. 1993. Recommendations for counseling persons infected with human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II). Ann. Intern. Med. 118:448–454.

59. Taylor, R. N. Report on CDC Performance Evaluation Testing. Consortium for Retrovirus Serology Standardization meeting entitled "An Update of HIV-1 and HTLV-I Serology" . 1988.
Ref Type: Presentation

60. Lal, RB. Delineation of immunodominant epitopes of human T-lymphotropic virus types I and II and their usefulness in developing serologic assays for detection of antibodies to HTLV-I and HTLV-II. Journal Acquired Immune Deficiency Syndrome Human Retrovirology. 1996. 13: S170-S178.

61. Tuppin, P., M. Makuwa, T. Guerma, M. M. Bazabana, J. C. Loukaka, D. Jeannel, P. M'Pele, and G. de The. Low HTLV-I/II seroprevalence in pregnant women in Congo and a geographic cluster of an HTLV-like indeterminate Western blot pattern. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 1996. 11: 105-107.

62. Bonis, J., P. M. Preux, L. Nzisabira, L. Letennueur, G. Muhirwa, T. Buzingo, A. Kamuragiye, C. Preux, E. Ngoga, M. Dumas, *et al*. HTLV-I in Burundi (east Africa): Lack of reactivity to the HTLV-I immunodominant envelope epitope. J. Acquir. Immune Defic. Syndr. 1994. 7: 1099-1100.

63. Gessain, A., R. Mahieux, and G. de The. 1995. HTLV-I "indeterminate" Western blot patterns observed in sera from tropical regions: the situation revisited. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 9: 316-319.

64. Hayes C.G., Burans JP., and Oberst RB. Antibodies to Human T Lymphotropic virus Type I in a population from the Philippines: Evidence for cross-reactivity with *Plasmodiun falciparum*. The J. Infect. Dis. 1990; 163: 257-262.

65. Lal, R. B., D. Rudolph, M. P. Alpers, A. J. Sulzer, Y. P. Shi, and A. A. Lai. 1994. Immunologic cross-reactivity between structural proteins of human T-cell lymphotropic virus type I and the blood stage *of Plasmodium falciparum*. Clin. Diagn. Lab. Immunol. 1: 5-10.

66. Porter, K. R., R. L. Anthony, A. Solihin, and C. G. Hayes. 1995. Mapping of a human T-lymphotropic virus type I gag protein epitope that cross-reacts with anti-*Plasmodium falciparum* antibodies. J. Med. Virol. 45: 469-474.

67. Porter, K. R., L. Liang, G. W. Long, M. J. Bangs, R. Anthony, E. M. Andersen, and C. G. Hayes. 1994. Evidence for anti-*Plasmodium falciparum* antibodies that cross-react with human T-lymphotropic virus type I proteins in a population in Iraian Jaya, Indonesia. Clin. Diagn. Lab. Immunol. 1: 11-15.

68. Mauclere, P., J. Y. Le Hesran, R. Mahieux, R. Salla, J. Mfoupouendoun, E. T. Abada, J. Millan, G. de The, and A. Gessain. 1997. Demographic, ethnic, and geographic differences between human T cell lymphotropic virus (HTLV) type I-seropositive carriers and persons with HTLV-I Gag-indeterminate Western blots in Central Africa. J. Infect. Dis. 176: 505-509

69. Lal, RB., Rudolph DL., Coligan JE., Brodine SK., and Roberts CR. Failure to detect evidence of human T-lymphotropic virus (HTLV) type I and type II in blood donors with isolated gag antibodies to HTLV-I/II. Blood 1992; 80: 544-550.

70. Courouce, A. M., J. Pillonel, J. M. Lemaire, M. Maniez, and J. B. Brunet. 1993. Seroepidemiology of HTLV-I/II in universal screening of blood donations in France. AIDS 7: 841-847.

71. Hjelle B., Cyrus S., Swenson S., and Mills R. Serologic distinction between human T-lymphotropic virus (HTLV) type I and HTLV type II. Transfusion 1991; 31: 731-736.

72. Khabbaz, RF., Heneine W., Grindon A., Hartley TM., Shulman G., and Kaplan J. Indeterminate HTLV serologic results in U.S. blood donors: Are they due o HTLV-I or HTLV-II? J. Acquir. Immune Defic. Syndr. 1992; 5: 400-404.

73. Lipka, JJ., Young KK., Kwok SY., Reyes GR., Sninsky JJ, and Foung SK. Significance of human T-lymphotropic virus type I indeterminant serological findings among healthy individuals. Vox Sang. 1991; 61: 171-176.

74. Soldan SS., Graf MD., Waziri A., Flerlage AN., Robinson SM., Kawaninshi T, Leist TP., Lehky TJ., Levin MC., and Jacobson S. HTLV-I/II seroindeterminate western blot reactivity in a cohort of patients with neurological disease. J. Infect. Dis. 1999; 180: 685-694.

75. Kwok, S., J. J. Lipka, N. McKinney, D. E. Kellogg, B. Poiesz, S. K. Foung, and J. J. Sninsky. 1990. Low incidence of HTLV infections in random blood donors with indeterminate western blot patterns. Transfusion 30: 491-494.

76. Busch, M. P., M. Laycock, S. H. Kleinman, J. W. Wages, Jr., M. Calabro, J. E. Kaplan, R. F. Khabbaz, and C. G. Hollingsworth. 1994. Accuracy of supplementary serologic testing for human T-lymphotropic virus types I and II in US blood donors. Retrovirus Epidemiology Donor Study. Blood 83: 1143-1148.

77. Cowan, E. P., G. J. Nemo, A. E. Williams, R. K. Alexander, A. Vallejo, I. K. Hewlett, R. B. Lal, C. S. Dezzutti, D. Gallahan, K. George, B. A. Pancake, D. Zucker-Franklin, P. R. McCurdy and E. Tabor. 1999. Absence of human T-lymphotropic virus type I tax sequences in a population of normal blood donors in the Baltimore, MD/Washington, DC, area: Results from a multi-center study. Transfusion 39: 904-909.

78. Rouet F., Meertens L., Courouble G., Herrmann-Storck C., Pabingui R., Chancerel B., Abid A., Strobel M., Mauclere P., and Gessain A. Serological, epidemiological, and molecular differences between human T-cell lymphotropic virus type I – seropositive healthy carriers and persons with HTLV-I gag indeterminate Western blot patterns from the Caribbean. J. Clin. Microbiol. 2001; 39: 1247-1253

79. Lal, R. B., J. J. Lipka, S. K. Foung, K. G. Hadlock, G. R. Reyes, and W. P. Carney. 1993. Human T lymphotropic virus type I/II in lake Lindu Valley Central Sulawesi, Indonesia. J. Acquir. Immune Defic. Syndr. 6: 1067-1068.

80. Mahieux, R., P. Horal, P. Mauclere, O. Mercereau-Puijalon, M. Guillotte, L Meertens, E. Murphy, and A Gessain. 2000. Human T-Cell Lymphototropic virus type 1 gag indeterminate Western blot patterns in central Africa: relationship to *Plasmodium falciparum* infection. J. Clin. Microbiol. 38: 4049-4057.

81. Lal, RB, Brodine, SK, Coligan, JE, and CR Roberts. Differential antibody responsiveness to p19 *Gag* results in serological discrimination between human T lymphotropic virus type I and type II. Journal of Medical Virology. 1991. V5 (4): 232-236

16. APPENDICES

a) Appendix 1: Known Positive Population Results

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1812	GD21, p24**,** rgp46-II	HTLV -II	4.37/4.89/4.70	HTLV-I (1:64) HTLV-II (1:1024)			HTLV -II
1813	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	16.42/17.94/18.56	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1814	GD21, p24, rgp46-II	HTLV -II	2.91/2.23/2.69	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1815	GD21, p19, p24, rgp46-II	HTLV -II	6.84/6.77/6.71	HTLV-I (1:256) HTLV-II (1:1024)			HTLV -II
1816	GD21, p24, rgp46-II	HTLV -II	1.83/2.04/2.08	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1817	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	13.84/13.77/13.68	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1818	GD21, p24, rgp46-II	HTLV -II	1.38/1.02/1.09	HTLV-I (1:16) HTLV-II (1:256)			HTLV -II
1819	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	14.67/13.77/14.14	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1820	GD21, p19, p24, rgp46-II	HTLV -II	4.72/4.14/4.17	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1821	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	13.21/11.49/11.71	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1822	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	8.50/6.64/6.85	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1823	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	18.73/17.73/17.38	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1824	GD21, p19, p24, p53, rgp46-II	HTLV -II	14.59/13.04/13.24	HTLV-I (1:256) HTLV-II (1:1024)			HTLV -II
1825	GD21, p19, p24, p26, p28, p32, p36, p53, rgp46-I	HTLV -I	2.08/1.43/1.40	HTLV-I (1:256) HTLV-II (1:16)			HTLV-I
1826	GD21, p19, p24, p53, rgp46-II	HTLV -II	16.55/12.84/12.49	HTLV-I (1:1024) HTLV-II (1:4096)			HTLV -II
1827	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	7.83/7.47/7.27	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1828	GD21, p19, rgp46-I	HTLV-I	2.23/1.16/1.24	HTLV-I (1:128) HTLV-II (1:64)			HTLV-I
1829	GD21, p24, rgp46-II	HTLV -II	4.31/3.09/3.96	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1830	GD21, p19, p24, rgp46-II	HTLV -II	4.16/3.34/3.55	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1831	GD21, p19, p24, p53, rgp46-II	HTLV -II	8.25/7.11/6.96	HTLV-I (1:256) HTLV-II (1:1024)			HTLV -II

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1832	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-II, rgp46-I	HTLV –I/II	15.48/14.60/ 14.57	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1833	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	5.77/5.25/5.8 2	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1834	GD21, p24, rgp46-II	HTLV -II	4.74/4.32/4.4 4	HTLV-I (1:16) HTLV-II (1:64)			HTLV -II
1835	GD21, p19, p24, rgp46-II	HTLV -II	4.92/3.82/4.1 7	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1836	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	18.50/17.47/ 19.20	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1837	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	23.42/22.87/ 23.23	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1838	GD21, p19, p24, p53, rgp46-II	HTLV -II	15.58/14.05/ 14.68	HTLV-I (1:1024) HTLV-II (1:4096)			HTLV -II
1839	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	9.66/11.30/1 1.90	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1840	GD21, p19, p24, p53, rgp46-II	HTLV -II	8.42/8.21/8.5 7	HTLV-I (1:256) HTLV-II (1:1024)			HTLV -II
1841	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-II, rgp46-I	HTLV –I/II	9.62/9.30/9.4 8	HTLV-I (1:512) HTLV-II (1:256)			HTLV-I
1842	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	20.28/18.53/ 18.89	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1843	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	4.19/3.27/3.8 5	HTLV-I (1:64) HTLV-II (1:8)			HTLV-I
1844	GD21, p19, p24, rgp46-II, rgp46-I	HTLV –I/II	1.80/1.60/1.6 8	HTLV-I (1:16) HTLV-II (1:64)			HTLV -II
1845	GD21, p19, p24, rgp46-II	HTLV -II	0.96				NEG
1846	GD21, p24, rgp46-II	HTLV -II	3.02/2.47/2.5 4	HTLV-I (1:16) HTLV-II (1:64)			HTLV -II
1847	GD21, p19, p24, p53, rgp46-II	HTLV -II	15.66/14.53/ 14.25	HTLV-I (1:256) HTLV-II (1:1024)			HTLV -II
1848	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	17.73/18.30/ 18.00	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1849	GD21, p24, rgp46-II	HTLV -II	2.52/2.47/2.5 3	HTLV-I (1:16) HTLV-II (1:64)			HTLV -II
1850	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	17.23/14.54/ 14.29	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1851	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	15.62/13.16/ 14.33	HTLV-I (1:2048) HTLV-II (1:512)			HTLV-I
1852	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	20.24/21.22/ 22.29	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1853	GD21, p24, rgp46-II	HTLV -II	2.20/2.57/2.6 5	HTLV-I (1:16) HTLV-II (1:256)			HTLV -II
1854	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	21.24/21.97/ 22.23	HTLV-I (1:2048) HTLV-II (1:1024)			HTLV-I
1855	GD21, p19, p24, p26, p28, p32, p36, rgp46-I	HTLV -I	2.17/1.86/2.0 1	HTLV-I (1:64) HTLV-II (1:16)			HTLV-I

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1856	GD21, p19, p24, p53, rgp46-II	HTLV -II	10.68/10.84/ 10.20	HTLV-I (1:64) HTLV-II (1:1024)			HTLV -II
1857	GD21, p19, p24, p26, p28, p32, p36, gp46, p53 rgp46-I	HTLV -I	15.38/15.11/ 15.36	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1858	GD21, p19, p24, p53, rgp46-II	HTLV -II	7.53/8.90/9.2 2	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1859	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV -I	4.52/4.15/4.6 4	HTLV-I (1:64) HTLV-II (1:16)			HTLV-I
1860	GD21, p19, p24, p26, p28, p32, p36, gp46, p53rgp46-I	HTLV -I	15.52/13.79/ 13.96	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1861	GD21, p19, p24, p26, p36, rgp46-I	HTLV -I	1.43/1.30/1.4 1	HTLV-I (1:32) HTLV-II (1:16)			HTLV-I
1862	GD21, p19, p24, p53, rgp46-II	HTLV -II	5.79/5.12/5.8 2	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1863	GD21, p19, p24, p26, p28, p32, p36	HTLV -I	2.05/1.83/2.2 2	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1864	GD21, p24, rgp46-II	HTLV -II	0.83				NEG
1865	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	21.66/17.68/ 17.48	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1866	GD21, p19, p24	HTLV -I	1.08/1.29/1.4 1	HTLV-I (1:8) HTLV-II (1:32)			HTLV -II
1867	GD21, p19, p24, rgp46-II	HTLV -II	4.20/4.66/4.8 6	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1868	GD21, p24, rgp46-II	HTLV -II	3.51/3.68/3.8 1	HTLV-I (1:8) HTLV-II (1:32)			HTLV -II
1869	GD21, p19, p24, p53, rgp46-II	HTLV -II	5.24/5.99/6.1 2	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1870	GD21, p19, p24, p53, rgp46-II	HTLV -II	13.93/12.74/ 12.97	HTLV-I (1:256) HTLV-II (1:1024)			HTLV –II
1871	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	21.89/17.07/ 17.98	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1872	GD21, p19, p24, p53, rgp46-II	HTLV -II	10.09/10.30/ 10.46	HTLV-I (1:1024) HTLV-II (1:4096)			HTLV –II
1873	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	8.61/7.04/7.9 7	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1874	GD21, p24, rgp46-II	HTLV -II	1.88/1.96/1.9 9	HTLV-I (1:16) HTLV-II (1:64)			HTLV –II
1875	GD21, p19, p24, p26, p28, p32, p36, GP46, p53, rgp46-I	HTLV -I	5.70/5.03/5.4 6	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1876	GD21, p19, p24, rgp46-II	HTLV -II	4.08/3.66/3.8 0	HTLV-I (1:64) HTLV-II (1:256)			HTLV -II
1877	GD21, p19, p24, rgp46-II	HTLV -II	8.29/8.83/8.6 9	HTLV-I (1:256) HTLV-II (1:1024)			HTLV -II
1673	GD21, p19, p24, p53, rgp46-II	HTLV-II	8.05/8.42/9.2 1	HTLV-I (1: 256) HTLV-II (1:1024)			HTLV-II
1674	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	20.79/24.58/ 25.02	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1675	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	16.00/21.03/ 21.34	HTLV-I (1:512) HTLV-II (1:256)			HTLV-I

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1676	GD21, p24	IND	2.31/2.06/2.25	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1677	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	20.64/16.15/16.64	HTLV-I (1:4096) HTLV-II (1:2048)			HTLV-I
1678	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	21.36/21.68/21.56	HTLV-I (1:4096) HTLV-II (1:2048)			HTLV-I
1679	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	14.55/13.28/14.18	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1680	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	13.01/12.59/13.38	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1681	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	10.78/9.56/9.94	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1682	GD21, p24	IND	1.90/2.38/2.30	HTLV-I (1:16) HTLV-II (1:64)			HTLV-II
1683	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	9.78/9.06/9.27	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1684	GD21, p19, p24, p36, rgp46-I	HTLV-I	4.98/4.58/4.86	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1685	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	24.64/26.48/28.65	HTLV-I (1:16384) HTLV-II (1:8192)			HTLV-I
1686	GD21, p24, rgp46-II	HTLV-II	2.85/3.33/3.56	HTLV-I (1:64) HTLV-II (1:128)			HTLV-II
1687	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	4.80/4.49/4.62	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1688	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	12.07/12.88/13.33	Inconclusive	p19, p28, p36, p21e, p53	Non-reactive	IND
1689	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	10.64/10.84/10.51	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1690	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	11.12/11.39/11.31	HTLV-I (1:1024) HTLV-II (1:4096)			HTLV-II
1691	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	11.64/11.10/11.19	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1692	GD21, p24, rgp46-II	HTLV-II	2.99/2.78/2.97	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1693	GD21, p24, p53, rgp46-II	HTLV-II	4.48/4.64/4.19	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1694	GD21, p24, rgp46-II	HTLV-II	2.59/2.58/2.50	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1695	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	21.73/23.70/23.76	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1696	GD21, p24, rgp46-II	HTLV-II	1.06/1.10/1.11	HTLV-I (1:16) HTLV-II (1:64)			HTLV-II
1697	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	21.94/26.08/25.97	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1698	GD21, p19, p24, p26, p28, p36, rgp46-I	HTLV-I	1.23/1.24/1.26	HTLV-I (1:256) HTLV-II (1:128)			HTLV-I

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1699	GD21, p19, p24, p36, rgp46-II	HTLV-II	5.47/5.01/5.47	HTLV-I (1:128) HTLV-II (1:512)			HTLV-II
1700	GD21, p19, p24, rgp46-II	HTLV-II	6.43/7.36/7.14	HTLV-I (1:256) HTLV-II (1:512)			HTLV-II
1701	GD21	IND	3.74/3.83/4.03	Non-Reactive	p19		NEG
1702	GD21, p19, p24, p36, rgp46-II	HTLV-II	6.98/7.43/7.41	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1703		NEG	3.79/4.16/4.32	Non-Reactive	Non-Reactive		NEG
1704	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	17.28/19.43/20.23	HTLV-I (1:512) HTLV-II (1:256)			HTLV-I
1705	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	12.77/14.28/14.69	HTLV-I (1:256) HTLV-II (1:128)			HTLV-I
1706	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	9.54/10.62/10.75	HTLV-I (1:64) HTLV-II (1:16)			HTLV-I
1707	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	21.97/24.42/24.13	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1708	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	23.72/27.51/28.40	HTLV-I (1:8192) HTLV-II (1:4096)			HTLV-I
1709	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	10.88/10.96/11.23	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1710	GD21, p24, rgp46-II	HTLV-II	2.88/3.02/3.29	HTLV-I (1:16) HTLV-II (1:64)			HTLV-II
1711	GD21, p24, rgp46-II	HTLV-II	5.40/5.20/5.54	HTLV-I (1:16) HTLV-II (1:128)			HTLV-II
1712	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	14.65/15.14/16.08	HTLV-I (1:2048) HTLV-II (1:1024)			HTLV-I
1713	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	22.72/27.52/27.66	HTLV-I (1:16384) HTLV-II (1:8192)			HTLV-I
1714	GD21, p24, rgp46-II	HTLV-II	2.46/2.98/2.98	HTLV-I (1:16) HTLV-II (1:128)			HTLV-II
1715	GD21, p24, p36, p53, rgp46-II	HTLV-II	9.19/8.61/8.82	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1716	GD21, p19, p24, rgp46-II	HTLV-II	2.03/1.88/1.24	HTLV-I (1:8) HTLV-II (1:64)			HTLV-II
1718	GD21, p19, p24, p26, p28, p32, gp46, p53	HTLV-I	11.21/11.86/12.03	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1719	GD21, p24, rgp46-II	HTLV-II	4.50/3.38/4.18	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1720	GD21, p19, p24, p36, rgp46-II	HTLV-II	5.26/4.44/4.22	HTLV-I (1:64) HTLV-II (1:1024)			HTLV-II
1721	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	9.74/8.84/8.98	HTLV-I (1:256) HTLV-II (1:4096)			HTLV-II
1722	GD21, p19, p24, p26, p28, p32, p36, rgp46-I	HTLV-I	2.67/2.49/2.54	HTLV-I (1:256) HTLV-II (1:128)			HTLV-I
1723	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	8.61/7.26/8.17	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1724	GD21, p24, rgp46-II	HTLV-II	2.14/1.75/1.74	HTLV-I (1:8) HTLV-II (1:16)			HTLV-II
1725	GD21, p19, p24, rgp46-II	HTLV-II	3.01/2.44/2.80	HTLV-I (1:256) HTLV-II (1:512)			HTLV-II
1726	GD21, p19, p24, p26, rgp46-II	HTLV-II	2.64/2.13/2.59	HTLV-I (1:8) HTLV-II (1:16)			HTLV-II
1727	GD21, p24, p36, rgp46-II	HTLV-II	5.43/4.36/4.73	HTLV-I (1:64) HTLV-II (1:128)			HTLV-II
1728	GD21, p19, p24, p36, rgp46-II	HTLV-II	6.36/5.40/5.06	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1729	GD21, p19, p24, p36, rgp46-II	HTLV-II	3.57/2.85/3.07	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1730	GD21, p19, p24, rgp46-II	HTLV-II	2.90/2.55/2.74	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1731	GD21, p19, p24, rgp46-II	HTLV-II	2.15/1.64/1.91	HTLV-I (1:64) HTLV-II (1:1024)			HTLV-II
1732	GD21, p24, p36, rgp46-II	HTLV-II	1.57/1.36/1.40	HTLV-I (1:8) HTLV-II (1:16)			HTLV-II
1735	GD21, p24, p36, rgp46-II	HTLV-II	6.28/4.88/4.96	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1736	GD21, p19, p24, p36, rgp46-II	HTLV-II	4.96/4.26/4.50	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1737	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	20.46/18.95/ 20.21	HTLV-I (1:2048) HTLV-II (1:1024)			HTLV-I
1738	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	12.82/10.36/ 10.60	HTLV-I (1:1024) HTLV-II (1:4096)			HTLV-II
1739	GD21, p19, p24, p26, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	13.13/12.02/ 12.67	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1740	GD21, p19, p24, rgp46-II	HTLV-II	2.98/2.30/2.53	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1741	GD21, p24, p36, p53, rg46-II	HTLV-II	5.69/4.66/6.20	HTLV-I (1:16) HTLV-II (1:64)			HTLV-II
1742	GD21, p19, p24, p36, p53, rgp46-II	HTLV-II	5.05/4.09/4.23	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1743	GD21, p24, rgp46-II	HTLV-II	2.35/1.97/2.07	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1744	GD21, rgp46-I	IND	1.77/1.40/1.46	HTLV-I (1:16) HTLV-II (1:8)			HTLV-I
1745	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	6.54/5.73/5.90	HTLV-I (1:128) HTLV-II (1:64)			HTLV-I
1746	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	16.57/16.41/ 17.15	HTLV-I (1:1024) HTLV-II (1:512)			HTLV-I
1747	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	18.39/17.79/ 16.98	HTLV-I (1:512) HTLV-II (1:256)			HTLV-I
1748	GD21, p24, rgp46-II	HTLV-II	3.55/3.60/3.76	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1749	GD21, p19, p24,p36, rgp46-II	HTLV-II	7.21/6.68/7.07	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1750	GD21, p19, p24, rgp46-II	HTLV-II	14.80/12.98/ 13.00	HTLV-I (1:1024) HTLV-II (1:4096)			HTLV-II
1751	GD21, p24, rgp46-II	HTLV-II	3.89/3.43/3.38	HTLV-I (1:8) HTLV-II (1:16)			HTLV-II

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1752	GD21, p19, p24, rgp46-II	HTLV-II	3.95/3.71/3.74	HTLV-I (1:16) HTLV-II (1:256)			HTLV-II
1753	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	14.69/13.41/13.46	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1754	GD21,p19, p24, p28, p32, p36, rgp46-I	HTLV-I	4.14/3.95/4.01	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1755	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	14.34/13.74/13.61	HTLV-I (1:4096) HTLV-II (1:2048)			HTLV-I
1756	GD21, p19, p24, rgp46-II	HTLV-II	2.40/2.35/2.39	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1757	GD21,p19, p24, p28, p32, p36, rgp46-I	HTLV-I	3.19/3.14/3.14	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1758	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	12.05/11.70/11.93	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1759	GD21, p19, p24, p28, p32, p36, gp46, p53	HTLV-I	6.60/5.37/5.68	HTLV-I (1:64) HTLV-II (1:16)			HTLV-I
1760	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	9.99/8.60/9.34	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1761	GD21, p24, rgp46-II	HTLV-II	1.50/1.31/1.36	HTLV-I (1:16) HTLV-II (1:64)			HTLV-II
1762	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	16.25/20.74/20.47	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1763	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	19.31/14.69/15.82	HTLV-I (1:8192) HTLV-II (1:4096)			HTLV-I
1764	GD21, p19, p24, rgp46-II	HTLV-II	2.86/2.91/3.01	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1765	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	7.47/6.64/7.96	HTLV-I (1:512) HTLV-II (1:256)			HTLV-I
1766	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	19.36/21.13/20.28	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1767	GD21, p19, p24, rgp46-II	HTLV-II	2.53/2.80/2.47	HTLV-I (1:64) HTLV-II (1:1024)			HTLV-II
1768	GD21, p19, p24, rgp46-II	HTLV-II	13.52/10.03/10.65	HTLV-I (1:1024) HTLV-II (1:4096)			HTLV-II
1769	GD21, p19, p24, rgp46-II	HTLV-II	10.48/7.78/9.15	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1770	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	14.31/11.54/12.69	HTLV-I (1:1024) HTLV-II (1:512)			HTLV-I
1771	GD21, p19, p24, rgp46-II	HTLV-II	18.48/19.17/18.78	HTLV-I (1:4096) HTLV-II (1:16384)			HTLV-II
1772	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	21.29/21.34/20.78	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1773	GD21, p24, rgp46-II	HTLV-II	4.46/3.94/4.17	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1774	GD21, p24, rgp46-II	HTLV-II	1.18/1.15/1.25	HTLV-I (1:16) HTLV-II (1:64)			HTLV-II
1775	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	25.04/27.19/26.06	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1776	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	13.25/11.45/11.03	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1777	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	18.34/19.08/18.69	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1778	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	12.15/10.60/11.65	HTLV-I (1:512) HTLV-II (1:256)			HTLV-I
1779	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-II, rgp46-I	HTLV-I/II	14.45/14.78/15.28	HTLV-I (1:512) HTLV-II (1:256)			HTLV-I
1780	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	14.13/14.32/13.75	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1781	GD21, p24, rgp46-II	HTLV-II	3.07/2.98/2.91	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1782	GD21, p24, rgp46-II	HTLV-II	2.54/2.15/2.27	HTLV-I (1:64) HTLV-II (1:256)			HTLV-II
1783	GD21, p19, p24, rgp46-II	HTLV-II	5.94/7.15/7.41	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1784	GD21, p19, p24, gp46, p53, rgp46-II	HTLV-II	9.00/8.87/8.97	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1785	GD21, p19, p24, p36, rgp46-II	HTLV-II	15.63/14.83/14.40	HTLV-I (1:1024) HTLV-II (1:4096)			HTLV-II
1786	GD21, p19, p24, p36, rgp46-II	HTLV-II	10.85/9.85/10.03	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1787	GD21, p19, p24, rgp46-II	HTLV-II	13.50/13.19/12.69	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1788	GD21, p24, rgp46-II	HTLV-II	2.80/2.69/2.83	HTLV-I (1:16) HTLV-II (1:256)			HTLV-II
1789	GD21, p19, p24, rgp46-II	HTLV-II	8.84/9.14/8.89	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1791	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	17.25/18.39/18.15	HTLV-I (1:2048) HTLV-II (1:1024)			HTLV-I
1792	GD21, p24, rgp46-II	HTLV-II	0.56				NEG
1793	GD21, p19, p24, p36, rgp46-II	HTLV-II	2.59/2.49/2.80	HTLV-I (1:64) HTLV-II (1:1024)			HTLV-II
1794	GD21, p19, p24, rgp46-II	HTLV-II	4.64/4.31/4.17	HTLV-I (1:64) HTLV-II (1:1024)			HTLV-II
1795	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	24.15/25.94/26.27	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1796	GD21, p19, p24, rgp46-II	HTLV-II	4.44/3.78/3.93	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1797	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	23.38/24.85/ 25.26	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1798	GD21, p19, rgp46-I	HTLV-I	3.59/3.52/3.7 2	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1799	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	22.41/24.18/ 23.65	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1800	GD21, p19, p24, rgp46-II	HTLV-II	1.60/1.80/1.8 6	Non-reactive	P24		NEG
1801	GD21, p19, p24, p26, p28, p32, p36,rgp46-II	HTLV-II	5.59/5.55/5.7 2	HTLV-I (1:16) HTLV-II (1:256)			HTLV-II
1803	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	13.66/13.35/ 14.73	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1804	GD21, p19, p24, p36, rgp46-II	HTLV-II	11.44/10.77/ 10.57	HTLV-I (1:256) HTLV-II (1:1024)			HTLV-II
1805	GD21, p19, p24	HTLV-II	1.36/1.31/1.4 7	HTLV-I (1:16) HTLV-II (1:256)			HTLV-II
1806	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	23.53/26.66/ 25.91	HTLV-I (1:4096) HTLV-II (1:1024)			HTLV-I
1807	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	15.55/16.34/ 16.91	HTLV-I (1:1024) HTLV-II (1:256)			HTLV-I
1808	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	20.59/22.34/ 23.05	HTLV-I (1:2048) HTLV-II (1:1024)			HTLV-I
1809	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	9.47/10.65/1 0.98	HTLV-I (1:256) HTLV-II (1:64)			HTLV-I
1810	GD21, p19, p24, rgp46-II	HTLV-II	6.87/7.76/7.6 8	HTLV-I (1:64) HTLV-II (1:1024)			HTLV-II
1811	GD21, p19, p24, p28, p32, p36, gp46, p53, rgp46-I	HTLV-I	23.38/26.34/ 26.56	HTLV-I (1:4096) HTLV-II (1:2048)			HTLV-I

b) Appendix 2: HTLV Screening Negative Population Results

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2014		NEG	0.57				NEG
2015		NEG	0.38				NEG
2016		NEG	0.41				NEG
2017	GD21	IND	0.33				NEG
2018		NEG	2.73/3.28/3.53	Nonreactive	Nonreactive		NEG
2019		NEG	0.27				NEG
2020		NEG	0.24				NEG
2021	GD21	IND	0.33				NEG
2022		NEG	0.27				NEG
2023		NEG	0.47				NEG
2024	GD21	IND	0.34				NEG
2025		NEG	0.59				NEG
2026	P19	NEG	0.49				NEG
2027		NEG	0.30				NEG
2028		NEG	0.35				NEG
2029	GD21	IND	0.66				NEG
2030	GD21	IND	0.27				NEG
2031		NEG	0.45				NEG
2032	rgp46-I, rgp46-II	IND	0.83				NEG
2033		NEG	0.33				NEG
2034		NEG	0.33				NEG
2035		NEG	0.76				NEG
2036		NEG	0.91				NEG
2037		NEG	0.80				NEG
2038		NEG	1.02/1.22/1.27	Nonreactive	Nonreactive		NEG
2039	GD21	IND	0.26				NEG
2040		NEG	0.57				NEG
2041		NEG	1.57/1.38/1.46	Nonreactive	Nonreactive		NEG
2042	GD21	IND	0.44				NEG
2043	GD21	IND	0.44				NEG
2044		NEG	0.37				NEG
2045		NEG	2.29/2.26/2.36	Nonreactive	Nonreactive		NEG
2046		NEG	0.45				NEG
2047		NEG	0.33				NEG
2048		NEG	0.18				NEG
2049		NEG	0.51				NEG
2050		NEG	0.43				NEG
2051		NEG	0.35				NEG
2052	GD21	IND	0.22				NEG
2053		NEG	0.30				NEG
2054		NEG	0.29				NEG
2055	GD21	IND	1.10/0.89/1.00	Nonreactive	Nonreactive		NEG
2056	GD21, rgp46-II	IND	0.50				NEG
2057		NEG	0.43				NEG
2058		NEG	0.36				NEG
2059		NEG	0.37				NEG
2060	GD21	IND	0.26				NEG
2061		NEG	0.45				NEG
2062	GD21, p24	IND	0.39				NEG
2063	GD21	IND	0.49				NEG
2064	p24	NEG	0.24				NEG
2065	GD21	IND	0.37				NEG
2066		NEG	0.46				NEG
2067		NEG	0.16				NEG
2068		NEG	0.34				NEG
2069		NEG	0.35				NEG
2070		NEG	0.36				NEG
2071		NEG	0.79				NEG
2072	GD21	IND	0.35				NEG

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2073		NEG	0.54				NEG
2074	GD21	IND	0.43				NEG
2075		NEG	11.20/9.76/8.02	Nonreactive	Nonreactive		NEG
2076		NEG	0.44				NEG
2077		NEG	0.93				NEG
2078		NEG	0.32				NEG
2079		NEG	1.63/1.83/1.81	Nonreactive	p21e	Nonreactive	NEG
1878		NEG	0.27				NEG
1879		NEG	0.20				NEG
1880		NEG	0.59				NEG
1881	p24	NEG	0.78				NEG
1882		NEG	0.30				NEG
1883	p19	NEG	0.41				NEG
1884		NEG	0.43				NEG
1885		NEG	0.26				NEG
1886		NEG	0.26				NEG
1887		NEG	0.53				NEG
1888		NEG	0.66				NEG
1889		NEG	0.35				NEG
1890		NEG	0.60				NEG
1891		NEG	0.39				NEG
1892	p24	NEG	0.32				NEG
1893		NEG	0.30				NEG
1894		NEG	0.53				NEG
1895		NEG	0.26				NEG
1896	p24	NEG	0.93				NEG
1897		NEG	0.31				NEG
1898	p24	NEG	0.82				NEG
1899		NEG	0.28				NEG
1900		NEG	0.21				NEG
1901		NEG	0.32				NEG
1902	GD21	IND	1.21/1.10/1.16	Non-Reactive	Non-Reactive		NEG
1903	GD21	IND	0.53				NEG
1904	rgp46-II	IND	0.38				NEG
1905	GD21, p24	IND	0.27				NEG
1906		NEG	0.19				NEG
1907		NEG	0.36				NEG
1908		NEG	0.21				NEG
1909		NEG	0.24				NEG
1910		NEG	0.28				NEG
1911		NEG	0.14				NEG
1912		NEG	0.66				NEG
1913	GD21	IND	0.32				NEG
1914		NEG	0.36				NEG
1915		NEG	0.60				NEG
1916		NEG	0.61				NEG
1917		NEG	0.61				NEG
1918		NEG	0.78				NEG
1919		NEG	0.27				NEG
1920		NEG	1.05/1.32/1.30	Non-Reactive	p24		NEG
1921		NEG	0.25				NEG
1922		NEG	0.67				NEG
1923		NEG	0.27				NEG
1924		NEG	0.28				NEG
1925		NEG	0.20				NEG
1926		NEG	0.28				NEG
1927		NEG	0.23				NEG
1928		NEG	0.26				NEG
1929		NEG	0.33				NEG
1930		NEG	0.26				NEG
1931		NEG	0.32				NEG
1932		NEG	0.87				NEG

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1933		NEG	0.21				NEG
1934	p19	NEG	0.26				NEG
1935		NEG	0.26				NEG
1936		NEG	0.29				NEG
1937		NEG	0.27				NEG
1938		NEG	0.33				NEG
1939		NEG	0.24				NEG
1940		NEG	0.56				NEG
1941		NEG	0.43				NEG
1942		NEG	0.36				NEG
1943	GD21	IND	0.01				NEG
1944		NEG	0.40				NEG
1945	GD21	IND	0.32				NEG
1946		NEG	0.23				NEG
1947		NEG	0.74				NEG
1948		NEG	0.21				NEG
1949		NEG	0.56				NEG
1950		NEG	0.54				NEG
1951	rgp46-I, rgp46-II	IND	0.21				NEG
1952		NEG	0.27				NEG
1953	rgp46-II	IND	0.27				NEG
1954	rgp46-II	IND	0.26				NEG
1955		NEG	0.48				NEG
1956		NEG	0.26				NEG
1957	p19	NEG	10.00/7.59/7.77	Non-reactive	Non-reactive		NEG
1958		NEG	1.05/0.56/0.58				NEG
1959	GD21, p24	IND	0.32				NEG
1960	rgp46-II	IND	0.30				NEG
1961		NEG	0.43				NEG
1962		NEG	0.39				NEG
1963		NEG	0.31				NEG
1964		NEG	1.99/2.38/2.31	Non-reactive	Non-reactive		NEG
1965		NEG	0.31				NEG
1966		NEG	0.34				NEG
1968		NEG	0.24				NEG
1969		NEG	0.46				NEG
1970		NEG	0.36				NEG
1971		NEG	0.45				NEG
1972		NEG	0.29				NEG
1973		NEG	0.34				NEG
1974		NEG	0.33				NEG
1975		NEG	0.30				NEG
1977		NEG	0.22				NEG
1978		NEG	0.36				NEG
1979		NEG	1.29/0.96/1.03	Non-reactive	Non-reactive		NEG
1980	p24	NEG	0.32				NEG
1981		NEG	0.31				NEG
1982		NEG	0.30				NEG
1983		NEG	0.40				NEG
1984		NEG	0.33				NEG
1985		NEG	0.47				NEG
1986		NEG	0.28				NEG
1987		NEG	1.48/1.11/1.21	Non-reactive	Non-reactive		NEG
1988		NEG	0.33				NEG
1989		NEG	0.33				NEG
1990		NEG	1.24/0.91/1.10	Non-reactive	P21E	Non-reactive	NEG
1991		NEG	0.53				NEG
1992		NEG	0.37				NEG
1993		NEG	0.21				NEG
1994		NEG	0.29				NEG
1995		NEG	0.42				NEG
1996		NEG	0.46				NEG

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
1997		NEG	0.31				NEG
1998		NEG	0.29				NEG
1999		NEG	0.36				NEG
2000		NEG	0.22				NEG
2001	rgp46-I	IND	0.17				NEG
2002		NEG	0.49				NEG
2003		IND	0.34				NEG
2004	p24	NEG	0.43				NEG
2005		NEG	0.33				NEG
2006		NEG	0.21				NEG
2007	p24	NEG	1.83/2.00/2.00	Non-reactive	P21E	Non-reactive	NEG
2008		NEG	0.44				NEG
2009		NEG	2.52/2.43/2.47	Non-reactive	Non-reactive		NEG
2010		NEG	0.33				NEG
2011		NEG	0.44				NEG
2012		NEG	0.47				NEG
2013		NEG	0.39				NEG

c) Appendix 3: HTLV Screening Reactive Population Results

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2080		NEG	0.75				NEG
2081	p24	NEG	0.79				NEG
2082	p19, p26, p28, p32, p36	NEG	1.12/1.04/1.08	Non-reactive	p19, p53	Non-reactive	NEG
2083	p19	NEG	0.75				NEG
2084	GD21	IND	1.42/1.29/1.93	Non-reactive	Non-reactive		NEG
2085	GD21	IND	1.56/1.47/1.64	Non-reactive	Non-reactive		NEG
2086		NEG	0.33				NEG
2087		NEG	1.88/1.86/1.67	Non-reactive	Non-reactive		NEG
2088		NEG	4.61/3.87/2.75	Non-reactive	p21e	Non-reactive	NEG
2089		NEG	0.47				NEG
2090		NEG	0.96				NEG
2091	GD21, rgp46-I	IND	1.14/1.09/1.06	Non-reactive	Non-reactive		NEG
2092		NEG	13.12/11.79/11.34	Non-reactive	Non-reactive		NEG
2093		NEG	0.69				NEG
2094		NEG	5.32/4.57/5.38	Non-reactive	Non-reactive		NEG
2095	p19, rgp46-II	IND	0.50				NEG
2096		NEG	1.46/1.14/1.09	Non-reactive	Non-reactive		NEG
2097	GD21	IND	0.57				NEG
2098	GD21	IND	0.97				NEG
2099	p19, p24, p26, p28, p32, p36	IND	0.75				NEG
2100	p19, p26, p28, p32, p36	NEG	0.79				NEG
2101	rgp46-II	IND	1.15/1.03/1.18	Non-reactive	Non-reactive		NEG
2102	GD21	IND	1.61/1.42/1.68	Non-reactive	Non-reactive		NEG
2103	p19, p26, p28, p32, p36	NEG	2.89/2.38/2.48	Non-reactive	p19	Non-reactive	NEG
2104		NEG	0.88				NEG
2105	GD21	IND	0.62				NEG
2106	GD21	IND	0.69				NEG
2107	p19	NEG	1.23/1.06/1.11	Non-reactive	p19	Non-reactive	NEG
2108	GD21	IND	0.53				NEG
2109	GD21, p53	IND	1.36/1.14/1.23	Non-reactive	p21e	Non-reactive	NEG
2110	p24	NEG	2.20/2.20/2.38	Non-reactive	Non-reactive		NEG
2111		NEG	2.88/2.34/2.66	Non-reactive	Non-reactive		NEG
2112	GD21	IND	7.56/6.82/7.44	Non-reactive	Non-reactive		NEG
2113		NEG	1.51/1.87/2.73	Non-reactive	Non-reactive		NEG
2114	GD21	IND	4.68/3.93/4.07	Non-reactive	Non-reactive		NEG
2115	GD21	IND	0.69				NEG
2116	p19	NEG	0.38				NEG
2117	p19, GP46, p53	IND	4.02/4.07/4.32	Non-reactive	Non-reactive		NEG

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2118		NEG	2.30/2.05/2.33	Non-reactive	Non-reactive		NEG
2119		NEG	0.63				NEG
2120	GD21	IND	0.80				NEG
2121	GD21, p24	IND	0.50				NEG
2122		NEG	1.28/0.90/1.19	Non-reactive	Non-reactive		NEG
2123		NEG	1.12/0.90/0.99				NEG
2124	rgp46-II	IND	2.96/2.95/3.06	Non-reactive	Non-reactive		NEG
2125		NEG	1.17/0.89/0.78				NEG
2126	p19, p26, p28, p32, p36, p53	NEG	2.05/1.79/1.82	Non-reactive	p19, p53	Non-reactive	NEG
2127	GD21	IND	1.08/0.83/0.99				NEG
2129	p19	NEG	0.33				NEG
2130	p24	NEG	3.31/3.50/3.54	Non-reactive	p24	Non-reactive	NEG
2131	GD21	IND	0.95				NEG
2132		NEG	0.70				NEG
2133	p19, p26, p28, p32, p36, p53	NEG	2.62/2.56/2.98	Non-reactive	P19, p24	Non-reactive	NEG
2134		NEG	0.32				NEG
2135	p19	NEG	1.26/1.06/1.17	Non-reactive	P19, p24	Non-reactive	NEG
2136	GD21, gp46	IND	0.65				NEG
2137	GD21	IND	0.98				NEG
2138		NEG	1.30/1.41/1.59	Non-reactive	Non-reactive		NEG
2139		NEG	0.98				NEG
2140	GD21	IND	1.11/0.94/1.10	Non-reactive	Non-reactive		NEG
2141		NEG	0.47				NEG
2142		NEG	0.31				NEG
2143		NEG	1.34/1.33/1.48	Non-reactive	Non-reactive		NEG
2144		NEG	3.52/3.33/3.48	Non-reactive	Non-reactive		NEG
2145	**GD21, p24, rgp46-II, rgp46-I**	**HTLV-II**	**1.02/1.10/1.21**	**Non-reactive**	**Non-reactive**		**NEG**
2146		NEG	0.90				NEG
2215	GD21	IND	0.37				NEG
2216	p19, p26, p28, p32, p36	NEG	0.73				NEG
2217		NEG	1.49/2.00/0.88	Non-reactive	Non-reactive		NEG
2218		NEG	0.55				NEG
2219		NEG	0.76				NEG
2220	GD21, p24	IND	1.73/1.76/1.90	Non-reactive	Non-reactive		NEG
2221	p19	NEG	0.41				NEG
2222		NEG	1.94/1.90/2.07	Non-reactive	Non-reactive		NEG
2223	GD21	IND	0.82				NEG
2224		NEG	2.43/2.50/3.34	Non-reactive	Non-reactive		NEG
2255		NEG	2.26/2.24/2.58	Non-reactive	Non-reactive		NEG
2256	GD21, p19	IND	2.61/2.93/3.03	Non-reactive	Non-reactive		NEG

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2257	GD21	IND	0.96				NEG
2258	GD21	IND	3.36/1.36/2.78	Non-reactive	Non-reactive		NEG
2259		NEG	1.24/1.43/1.43	Non-reactive	Non-reactive		NEG
2260	GD21	IND	5.01/3.92/4.74	Non-reactive	Non-reactive		NEG
2261	GD21	IND	1.07/0.92/0.87				NEG
2262	p19, p26, p28, p32, p36	NEG	1.99/2.01/2.34	Non-reactive	p19		NEG
2263	GD21	IND	1.86/2.33/2.86	Non-reactive	Non-reactive		NEG
2264	GD21	IND	1.36/1.29/1.35	Non-reactive	Non-reactive		NEG
2265	p19, p26, p28, p32, p36	NEG	3.08/3.44/3.60	Non-reactive	p19		NEG
2266		NEG	0.83				NEG
2267	p19, p26, p28, p32, p36	NEG	0.56				NEG
2268		NEG	2.37/2.37/2.47	Non-reactive	Non-reactive		NEG
2269	p19, p26, p28, p32, p36	NEG	2.15/2.21/2.58	Non-reactive	p19		NEG
2270	GD21, gp46-II	IND	1.20/1.08/1.23	Non-reactive	Non-reactive		NEG
2271		NEG	1.21/1.29/1.45	Non-reactive	Non-reactive		NEG
2272	GD21, rgp46-II, rgp46-I	IND	2.49/2.22/2.99	Non-reactive	Non-reactive		NEG
2273	GD21	IND	1.32/1.40/1.41	Non-reactive	Non-reactive		NEG
2275		NEG	1.67/1.49/1.08	Non-reactive	Non-reactive		NEG
2276	p19, p26	NEG	1.37/1.45/1.69	Non-reactive	p19		NEG
2277	GD21	IND	0.83				NEG
2278	GD21, p19	IND	0.74				NEG
2279	p19, p26, p28, p36	NEG	0.83				NEG
2280		NEG	10.23/10.50/10.78	Non-reactive	Non-reactive		NEG
2281	p19, p26, p28, p32, p36	NEG	3.77/3.92/4.10	Non-reactive	p19, p24, p36, p21E, p53	Non-reactive	IND
2282	GD21, rgp46-II, rgp46-I	IND	0.80				NEG
2283	p19, p26, p28, p32, p36	NEG	1.42/1.55/1.49	Non-reactive	p19, p24		NEG
2284	p19, p26, p28, p32, p36	NEG	2.78/2.58/3.33	Non-reactive	p19, p24		NEG
2285	GD21	IND	0.73				NEG
2286	p19, rgp46-II	IND	3.21/2.35/3.47	Non-reactive	p19		NEG
2287	p19	NEG	1.28/1.36/1.66	Non-reactive	Non-reactive		NEG
2288	GD21, p19	IND	4.85/4.41/3.85	Non-reactive	Non-reactive		NEG
2289	GD21, rgp46-II, rgp46-I	IND	1.55/1.97/2.12	Non-reactive	P24		NEG
2290	p19, p26, p28, p32, p36	NEG	1.30/1.31/1.54	Non-reactive	p19, p24		NEG
2291	GD21, p24	IND	0.41				NEG
2293		NEG	2.16/2.24/2.56	Non-reactive	Non-reactive		NEG

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2294	GD21	IND	0.62				NEG
2295	GD21, p24	IND	1.05/0.90/0.80				NEG
2296	p19, p24, p26, p28, p32, 36	IND	1.34/1.58/1.71	Non-reactive	p19, p24		NEG
2297	p19, p26, p28, p32, 36	NEG	4.42/4.57/4.93	Non-reactive	p19, p24		NEG
2298	p19, rgp46-II	IND	0.78				NEG
2299	p26	NEG	1.43/1.64/1.73	Non-reactive	Non-reactive		NEG
2300		NEG	3.63/3.75/3.26	Non-reactive	Non-reactive		NEG
2301	GD21	IND	1.06/1.11/1.27	Non-reactive	Non-reactive		NEG
2302		NEG	3.37/3.19/3.51	Non-reactive	Non-reactive		NEG
2303	p19, p26, p28, p32, p36, p53	NEG	3.00/3.58/3.58	Non-reactive	p19, p24		NEG
2304	p19	NEG	2.41/2.52/2.22	Non-reactive	p19		NEG
2305	GD21	IND	0.83				NEG
2306		NEG	1.28/1.62/1.86	Non-reactive	Non-reactive		NEG
2307	p19, p26, p28, p32, p36	NEG	1.27/1.57/1.66	Non-reactive	p19, p24, p21E	Non-reactive	IND
2308	GD21	IND	0.92				NEG
2309		NEG	0.56				NEG
2310	GD21	IND	0.53				NEG
2311		NEG	1.57/1.78/1.77	Non-reactive	Non-reactive		NEG
2312	GD21, p19	IND	0.75				NEG
2316		NEG	1.86/2.28/2.42	Non-reactive	Non-reactive		NEG
2147		NEG	2.65/2.41/2.53	Non-reactive	Non-reactive		NEG
2148	**GD21, p19, p24, rgp46-I**	**HTLV-I**	**1.03/0.95/1.03**	**Non-reactive**	**p19**		**NEG**
2149	GD21	IND	1.98/1.66/1.83	Non-reactive	Non-reactive		NEG
2150		NEG	0.60				NEG
2151	p19, p36	NEG	0.76				NEG
2152		NEG	1.13/1.10/1.17	Non-reactive	Non-reactive		NEG
2153	**GD21, p19, p24, rgp46-I**	**HTLV-I**	**0.65**				**NEG**
2154		NEG	0.87				NEG
2155	**GD21, p19, p24, rgp46-II, rgp46-I**	**HTLV-I/II**	**4.36/2.97/2.43**	**Non-reactive**	**Non-reactive**		**NEG**
2157	GD21	IND	3.14/2.19/3.05	Non-reactive	Non-reactive		NEG
2158	GD21	IND	0.77				NEG
2159	**GD21, p19, p24, p36**	**HTLV-I**	**0.86**				**NEG**
2160	GD21	IND	2.67/2.56/2.70	Non-reactive	Non-reactive		NEG
2161		NEG	0.44				NEG
2162	GD21	IND	0.35				NEG
2163	GD21	IND	0.36				NEG
2164	GD21, p19, p26, p28, p32, p36	IND	0.56				NEG
2165		NEG	0.44				NEG
2166		NEG	2.58/2.22/2.67	Non-reactive	Non-reactive		NEG
2167	GD21	IND	0.56				NEG

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2168	GD21	IND	1.96/2.10/1.95	Non-reactive	Non-reactive		NEG
2169	GD21	IND	0.68				NEG
2170	**GD21, p19, p24, p26, p28, p32, p36, gp46, p53**	**HTLV-I**	**4.92/6.26/6.70**	**Non-reactive**	**p24, p21E**		**NEG**
2171	GD21	IND	1.64/1.85/1.87	Non-reactive	P21E	Non-reactive	NEG
2172		NEG	1.22/1.48/1.59	Non-reactive	Non-reactive		NEG
2173		NEG	6.96/8.48/8.87	Non-reactive	Non-reactive		NEG
2174		NEG	1.89/2.66/2.60	Non-reactive	Non-reactive		NEG
2175	GD21, p19, p26, p28, p32, p36, p53	IND	2.05/2.50/2.60	Non-reactive	p19		NEG
2176	***GD21, p19, rgp46-II, rgp46-I***	***IND[a]***	***0.61***				***NEG***
2177	GD21	IND	0.82				NEG
2178	GD21, p19, p26, p28, p32, p36	IND	0.66				NEG
2179	GD21	IND	0.88				NEG
2180	GD21, rgp46-I	IND	4.37/5.04/5.15	Non-reactive	Non-reactive		NEG
2181	GD21, rgp46-II, rgp46-I	IND	0.40				NEG
2182		NEG	1.68/1.73/1.99	Non-reactive	Non-reactive		NEG
2183	GD21	IND	1.15/1.23/1.53	Non-reactive	Non-reactive		NEG
2184		NEG	11.05/11.93/12.39	Non-reactive	Non-reactive		NEG
2185		NEG	1.95/2.13/2.45	Non-reactive	Non-reactive		NEG
2186	p19, p26, p28, p32, p36	NEG	0.42				NEG
2187	GD21, rgp46-II	IND	0.42				NEG
2188	p19, p26, p28, p32, p36, rgp46-II	IND	0.52				NEG
2189	**GD21, p24, rgp46-II**	**HTLV-II**	**0.49**				**NEG**
2190	GD21, rgp46-I	IND	1.05/1.14/1.32	Non-reactive	Non-reactive		NEG
2191	GD21, rgp46-I	IND	0.31				NEG
2192	GD21	IND	0.37				NEG
2193	GD21, p19	IND	0.37				NEG
2194	GD21, p19	IND	1.32/1.68/1.71	Non-reactive	p19		NEG
2195	p19	NEG	0.28				NEG
2196	GD21	IND	1.17/1.74/2.00	Non-reactive	Non-reactive		NEG
2197	p19, p26, p28, p32, p36	NEG	1.15/1.49/1.45	Non-reactive	p19		NEG
2198	GD21, p19, rgp46-II	IND	0.68				NEG
2199	GD21,p19	IND	1.14/1.19/1.43	Non-reactive	p19		NEG
2200		NEG	3.15/3.49/3.86	Non-reactive	Non-reactive		NEG
2201	GD21, rgp46-II, rgp46-I	IND	0.44				NEG
2202	rgp46-II	IND	1.23/1.46/1.52	Non-reactive	P21E	Non-reactive	NEG
2203	GD21	IND	0.96				NEG

Sample ID	*MP Blot bands present*	*MP Final Call*	*CDPHL EIA*	*CDPHL IFA*	*CDPHL WB*	*CDPHL RIPA*	*CDPHL Final Result*
2204	GD21	IND	0.63				NEG
2205	rgp46-II, gp46-I	IND	0.44				NEG
2206		NEG	5.06/5.92/6.54	Non-reactive	Non-reactive		NEG
2207		NEG	0.40				NEG
2208	p19, p26, p28, p32, p36	NEG	0.98				NEG
2209		NEG	2.67/3.14/3.39	Non-reactive	Non-reactive		NEG
2210	GD21, p19	IND	0.60				NEG
2211	p19	NEG	1.20/1.52/1.59	Non-reactive	p19		NEG
2212	GD21, p19, p26, p28, p32, p36	IND	0.59				NEG
2213	p19, p26, p28, p32, p36	NEG	1.52/2.09/2.08	Non-reactive	p19, p28, p21E, p53	Non-reactive	IND
2214		NEG	4.76/5.97/5.87	Non-reactive	Non-reactive		NEG

[a] This sample was incorrectly resulted as Indeterminate. A band pattern of GD21, p19 and rgp46-I should result in a HTLV-I interpretation.